

Aus dem Institut für Pflanzenbau und Grünland  
der Universität Hohenheim  
Fachgebiet Spezieller Pflanzenbau  
Prof. Dr. W. Aufhammer

**Management of *Fusarium graminearum*-inoculated crop  
residues — Effects on head blight, grain yield and grain  
quality of subsequent winter wheat crops**

Dissertation  
zur Erlangung des Grades eines Doktors  
der Agrarwissenschaften  
vorgelegt der  
Fakultät III – Agrarwissenschaften I  
(Pflanzenproduktion und Landschaftsökologie)  
der Universität Hohenheim

von  
M.Sc. agr. Cuilin Yi  
aus  
Heilongjiang, China

2001

Die vorliegende Arbeit wurde am 12. September 2001 von der Fakultät III – Agrarwissenschaften I der Universität Hohenheim als “Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften” angenommen.

Tag der mündlichen Prüfung:

8.11.2001

Dekan:

Prof. Dr. R. Blaich

Berichterstatter, 1. Prüfer:

Prof. Dr. W. Aufhammer

Mitberichterstatter, 2. Prüfer:

Prof. Dr. H. Buchenauer

3. Prüfer:

Prof. Dr. V. Römheld

## Contents

<b>A. General introduction</b>	<b>1</b>
<b>B. Problem definition</b>	<b>4</b>
<b>C. Summary of material and methods</b>	<b>6</b>
<b>D. Results – Publications</b>	<b>8</b>
1. <b>Methodological problems in field investigations on <i>Fusarium graminearum</i> infection of wheat.</b> Journal of Plant Diseases and Protection, 2000, 107 (6): 583-593. Reprinted with permission from E. Ulmer Verlag.	<b>8</b>
2. <b>Populations of <i>Fusarium graminearum</i> on crop residues as affected by incorporation depth, nitrogen and fungicide application.</b> Journal of Plant Diseases and Protection. Submitted.	<b>19</b>
3. <b>Head blight (<i>Fusarium graminearum</i>) and deoxynivalenol concentration in winter wheat as affected by pre-crop, soil tillage and nitrogen fertilization.</b> Journal of Plant Diseases and Protection, 2001, 108 (3): 217-230. Reprinted with permission from E. Ulmer Verlag.	<b>36</b>
4. <b>Effects of residue management of <i>Fusarium graminearum</i> inoculated pre-crops on grain yield and quality of winter wheat.</b>	<b>50</b>
<b>E. Summary of main results</b>	<b>63</b>
<b>F. General discussion</b>	<b>65</b>
<b>G. Summary/Zusammenfassung</b>	<b>70</b>
<b>H. References</b>	<b>76</b>
<b>I. Acknowledgments</b>	<b>83</b>



## A. General introduction

*Fusarium* head blight (FHB), caused by *Fusarium* spp., has become one of the most destructive diseases of crops world wide. Among *Fusarium* spp., *F. graminearum* was proved the most aggressive species towards wheat ears during pathogenicity tests (MESTERHAZY 1978, STACK & MULLEN 1985; KIECANA 1987; KIECANA et al. 1988). In China, the USA, Canada, some European countries and also in South Germany, *F. graminearum* is the dominating *Fusarium* species. It attacks a wide range of plant species: cereals, beans, peas, sunflowers, tomatoes, soybeans and radishes (AGARWAL 1976), and affects all vegetative and reproductive organs of plants, causing seedling blight, crown rot, foot rot and head blight in cereals, especially in wheat.

The disease incidence could reduce kernel set and grain weight, thereby causing significant yield losses which range from 30% to 70% under epidemic conditions (MIEDANER 1997). Consequently, grains are contaminated with fungal metabolites, which are toxic for human beings and animals (SUTTON 1982; MIROCHA et al. 1989; TANAKA et al. 1990; JENKINSON & PARRY 1994; SCOTT et al. 1996; WANG 1996; MIEDANER 1997; DILL-MACKY & JONES 2000). *F. graminearum* produces a variety of mycotoxins, deoxynivalenol (DON) and its derivatives were most often found in wheat (TANAKA et al. 1988, 1990; MIROCHA et al. 1989; SCOTT et al. 1990, 1997). Of 114 isolates of *F. graminearum* collected from soil or cereals on a world-wide basis, 95% were capable of producing DON (MIROCHA et al. 1989). Approximately 50% of DON in naturally contaminated wheat remain after the milling process, in the flour DON is very stable during baking (BOYACIOGLU et al. 1993; DEXTER et al. 1996). In view of that, many countries have set legal thresholds for *Fusarium* toxin concentrations in food and feed, e. g. in Canada DON concentration  $< 2$  or  $< 5$  mg kg<sup>-1</sup>, in the USA  $< 1$  or  $< 5$ -10 DON mg kg<sup>-1</sup> in food or feed, respectively, and in Austria  $< 0.5$  DON mg kg<sup>-1</sup> in food (GILGENBERG-HARTUNG 1999). Besides the toxicological aspect, the germination percentage of infected grains is substantially reduced. With about 92% of infected grains, germination was below 20%, and the germination of severe shrivelled grains due to *Fusarium* infection was none (BECHTEL et al. 1985). Baking quality of flour from infested crops was also detracted from the grains of FHB. Reductions in protein content, falling number and sedimentation value were observed, and the loaf volume of bread was decreased also (BECHTEL et al. 1985; MEYER et al. 1986; DEXTER et al. 1996; MIEDANER 1997; NIGHTINGALE et al. 1999). Some authors, however, found that the protein content even increased with increasing infection levels (MEYER et al. 1986; PAWELZIK et al. 1998).

The pathogens of *F. graminearum* use infected host tissue for carryover (ATTANASOFF 1920, 1923). Infested residues may give rise directly to infectious mycelium, or the residues may serve as a food base for sporulation and

## 2 General introduction

dissemination. The infection can originate from conidia or from ascospores produced in perithecia on residues of preceding crops such as maize and wheat above ground where light is available (COOK 1968; NYVALL 1970). It is favoured by periods of relative humidity of >92% (COOK 1981) and temperatures of 18-30°C (SUTTON 1982; OBST 1988; BECK et al. 1993; PARRY et al 1995; WEINERT & WOLF 1995; SCHWEYDA 1996). An ear infection may occur at any time from heading to maturity, but FHB is most severe after infections in the flowering period (ANDERSON 1948a; DIEHL 1984; MIELKE 1988; GANG 1997). If the inoculum arrives before or after anthesis, infections may still occur, but the seriousness of the disease is greatly reduced (RINTELEN 1997).

Rainfall and wind are involved in the dispersal of *Fusarium* spores. STRAUSBAUGH & MALOY (1986) found that much more ears were infected with *F. graminearum* and other *Fusarium* spp. in wheat crops receiving overhead irrigation (89% ear infection) compared with crops which did not receive irrigation (0% ear infection). Obviously, irrigation played an important role in the dispersal of *Fusarium* inoculum, or in the infection process because it enhanced the air humidity. JENKINSON and PARRY (1994) assumed that conidia can pass distances of about 1 m by wind within field crops. WANG (1996) showed that the airborne *Fusarium* spores in cereal crops were mainly ascospores and that their incidence was closely related to rainfall events. The number of spores usually peaked on days with rain and 1-2 days afterwards. He found that ascospores were released actively only 1-2 cm above the soil surface but they can be transported across long distances by wind. The conidia may serve as water-splash inoculum to produce stem rot, head blight, or they may enter the soil where either they convert into chlamydospores (COOK 1968; NYVALL 1970) or lyse. The chlamydospores, formed either in soil or in host tissues, are the main survival structure of many *Fusaria*. (GRIFFIN 1981). Other authors suppose from field observations of disease occurrence that spores of *F. graminearum* can be disseminated by wind up to 50 m in height and 30 m in horizontal distance from the source (BECK et al. 1997; FERNANDO et al. 1997; LEPSCHY et al. 1997).

The efficient control of *F. graminearum* by fungicides up-to-date is not possible, yet, especially in epidemic years (FEHRMANN & DIEHL 1989; JORDAN & HUTCHEON 1989; MESTERHAZY 1997b). MILUS & PARSONS (1994) assume that some fungicides are effective only at low infection levels. Fungicides containing tebuconazole, e. g. Folicur, show efficacy against FHB since yields can be increased by 35% due to the application of the fungicide (FEHRMANN & DIEHL 1989; JORDAN & HUTCHEON 1989; MESTERHAZY 1997b). But the effect depends much on weather conditions (OBST et al. 1992). It may even happen that the application of fungicides which are not intended for FHB control is associated with increased DON accumulation in grain (HART & WARD 1997; HERMANN et al. 1999).

Cultural practices and crop husbandry can assist in reducing the ear infection with *Fusarium*, thus decreasing the subsequent accumulation of mycotoxins and increasing the grain yield and quality to some degree (ZENTNER et al. 1990; COX & SHELTON 1992; SNIJDERS 1994; BORGHI et al. 1995; LÓPEZ-BELLIDO et al. 1998; MILLER et al. 1998; DILL-MACKY & JONES 2000). The increased incidence of FHB in many regions world wide has been attributed to high percentages of wheat and maize in crop rotations, reduced tillage and prolonged residue retention on the soil surface (STURZ & JOHNSTON 1985; SUMMERELL et al. 1990; DILL-MACKY 1997; WANG 1997; MILLER et al. 1998; DILL-MACKY & JONES 2000). According to SEAMAN (1982) and BECK & LEPSCHY (2000), maize and wheat grown in rotation leave abundant residues which are a primary source of inoculum. Crop rotation or residue management may not only reduce the primary inoculum and FHB (SNIJDERS 1994; WANG 1996; MILLER et al. 1998), but also influence the grain yield and grain quality (ZENTNER et al. 1990; COX & SHELTON 1992; BORGHI et al. 1995; LÓPEZ-BELLIDO et al. 1998). BORGHI et al. (1995) found that the wheat grain yield from a maize-wheat-alfalfa rotation advanced about 25% in comparison with that from a monoculture and its rotation significantly increased protein content. There is considerable evidence that the management of *Fusarium*-infested crop residues can reduce FHB incidence and severity (COOK & BAKER 1983; KHONGA & SUTTON 1988; SUTTON & VYN 1990). DILL-MACKY & JONES (2000) found that FHB incidence and severity were lower in moldboard ploughed plots than in either chisel ploughed or no-tilled plots. Furthermore, with no-till operation compared to conventional tillage a lower grain protein content and effects on other grain quality traits were observed, although the dough quality was not impaired (LÓPEZ-BELLIDO et al. 1998).

SUTTON & VYN (1990) suggested that increasing concentrations of organic acids or other antifungal substances may act also to suppress the population of *Fusarium* to grow and develop on crop residues, thus reduce the FHB incidence of subsequent crops. Liming reduced the severity of *Fusarium* wilt (*F. oxysporum*) and nitrate-nitrogen applied in addition to calcium hydroxide decreased *Fusarium* wilt even more (WOLTZ & ENGELHARD 1972). The incidence and severity of *Fusarium* root-rot diseases of winter wheat was higher after fertilization with ammonium (NH<sub>4</sub>) compared with nitrate (NO<sub>3</sub>), and wheat fertilized with urea (CO(NH<sub>2</sub>)<sub>2</sub>) showed less FHB symptoms than wheat fertilized with ammonium nitrate (HUBER & WATSON 1965; SMILEY et al. 1972; TEICH 1987; MARTIN et al. 1991). Moreover, manure in combination with N - fertilizer could especially improve grain quality of wheat (BORGHI et al. 1995).

## 4 Problem definition

### B. Problem definition

*F. graminearum* epidemics cause extensive damage through losses in grain yield and grain contamination with mycotoxins. They are generally considered to originate from inoculum associated with infested crop residues mainly of maize and wheat. Reducing *Fusarium*-inoculated crop residues on the soil surface as well as measures to reduce *Fusarium* inoculum on residues may be a key to control this disease. In this context the objective of the present study was to answer the following questions:

1. *F. graminearum* is able to spread spores across large distances within crops. For plot experiments in the field with artificial inoculation, simulating the natural way of infection, it has to be considered whether individual treatments can be efficiently separated from each other by isolation strips. The first question to study was: Is it possible to prevent the dispersal of *F. graminearum* from inoculated plots to non-inoculated plots of wheat by isolation strips of tall-growing crops?
2. The increased incidence of FHB has been attributed to reducing tillage and led to more *Fusarium* infested residues left on the soil surface. *Fusarium* spp. fungi act as crop residue decomposers and feed on the residues. Hence, the inoculum density is reduced as stubble decomposes, but even under conditions favourable for decomposition the pathogen can survive for at least one year. In order to suppress *F. graminearum* growth and development on residues, we can either try a direct control by a fungicide treatment of the crop residues or we can try to force decomposition by residue placement and mineral nitrogen application. The question to this position was: What are the direct effects of a fungicide or the indirect effects of incorporation and additional nitrogen supply on the decomposition of crop residues and the population level of *F. graminearum* on the residues?
3. Maize and wheat grown in rotation leave abundant residues which are the primary source of inoculum. Reducing infested crop residues on the soil surface as well as measures to reduce inoculum of *Fusarium* on residues will assist in reducing both the disease incidence and DON contamination in grains. Tillage practice as well as fungistatic compounds, e. g. nitrolime, may suppress the population of *Fusarium* on crop residues, thus reducing FHB incidence and DON contamination. In this context the questions were: Does the FHB infection level and mycotoxin contamination of winter wheat crops differ after infected pre-crops of either maize or wheat? Can we control FHB infection and reduce DON contamination of winter wheat grains by reducing the crop residues of the pre-crops on the soil surface due to silage use or by ploughing under the residues of combined crops? Can nitrolime fertilization, acting also as a



fungistatic agent, help to reduce FHB infection and DON contamination of winter wheat grains in the field?

4. Besides the ear infection with *F. graminearum* and the DON contamination of grains, also a reduction in grain yield and grain quality, e. g. 1000-grain weight, protein content, sedimentation value and falling number, was observed during epidemic years. Crop residue management may not only reduce the primary inoculum and FHB incidence but also influence the grain yield and grain quality. Our question was: How does the residue management of maize and spring wheat and the resulting FHB infestation affect grain yield and processing quality of subsequent winter wheat?

### C. Summary of material and methods

To answer the different questions stated above, field and greenhouse experiments were conducted. Subsequently, the principles of material and methods of the different empirical approaches are described. For more details refer to the 4 publications, which present the results focused on the 4 questions. Each publication comprises individually an introduction and the relevant materials and methods.

#### 1. Isolation-strip field experiment

##### 1.1 Experimental design

A factorial field experiment was conducted at the experimental station Ihinger Hof of Hohenheim University in 1997/98 with winter wheat (cv. Flair) and 1998/99 with spring wheat (cv. Quattro), arranged in a complete randomised block design with 4 replicates. The treatments (T1-T3) were 2 m, 4 m and 8 m wide isolation-strips of tall growing winter rape crops (cv. Lirajet, 1997; cv. Express, 1998) separating non-inoculated test plots of wheat from inoculated wheat plots. The wheat crops on inoculated plots were artificially inoculated by *F. graminearum* infected oat grains.

##### 1.2 Observations and measurements

Fifty ears were sampled at random from each test plot three times at medium and late milk ripeness as well as early dough ripeness, respectively. From these samples both the number of infected ears and the number of infected spikelets were counted. Finally, the number of infected spikelets per infected ear was calculated.

#### 2. Residue management greenhouse test

##### 2.1 Experimental design

Samples (stems including internodes and nodes) collected from pre-crop maize and spring wheat of the main field experiment were cut to pieces (2 cm), dried and immediately used for greenhouse tests.

Each fiberglass-mesh bag was placed inside 5 g of residue dry matter and the bag was incorporated with soil mixture in pots at 5, 10 and 15 cm depth. After that four treatments were applied to the pots, *i.e.* T1: Control (only soil plus straw in bag); T2: Addition of calcium ammonium nitrate (CAN) equivalent to 200 kg N ha<sup>-1</sup>; T3: Addition of nitrolime equivalent to 200 kg N ha<sup>-1</sup>; T4: Straw treatment with the fungicide Folicur. After 30, 60 and 90 days the residues were recovered from the bags and the residual material was further analyzed. Each experimental unit was replicated 8 times for each sampling date.

## 2.2 Observations and measurements

The litter-bags were recovered from the pots at each sampling date (30, 60 or 90 days). Afterwards the dry weight of the remaining residues per bag were determined. The weight difference between the incorporated 5 g and the remaining residues is indicated as the decomposition of residues (g).

The Colony Forming Unit (CFU) defined as the number of colonies developing on a suitable agar medium was determined on the basis of 1 g dry matter of the recovered residues (SCHWEYDA 1996). The conidiospore density was determined from another 1 g sub-sample of remaining residue material per litter-bag under microscope.

## 3. Residue management field experiment

### 3.1 Experimental design

A factorial field experiment was conducted at the experimental station Ihinger Hof of the University of Hohenheim in 1997-99 in a split-plot design with four replicates. Different pre-crops were arranged on main plots with treatments on sub-plots. Maize (cv. Helix) or spring wheat (cv. Hanno) were planted as pre-crops in rotations with winter wheat, and they were inoculated by *F. graminearum* infected oat grains. After the harvest of the pre-crops, winter wheat (cv. Flair) was sown in October 1997 and November 1998, respectively.

There were four treatments (T1-T4) in the field experiment. Pre-crops were either harvested for silage (T1) or harvested for grain (T2-T4). Subsequently, the residues were either left on the soil surface (T1, T3, T4) or ploughed under (T2) before sowing the winter wheat. Nitrogen fertilizer was applied to winter wheat either with calcium ammonium nitrate (CAN, T1-T3) or 62.5% of CAN were substituted by nitrolime (T4).

### 3.2 Observations and measurements

The stem infection of the pre-crop maize and the ear infection of the pre-crop spring wheat were assessed at dough stage. The ear infection of the test crops winter wheat was studied four times at medium milk ripeness, at late milk ripeness, at early dough ripeness as well as at medium dough ripeness from random samples of 50 ears per plot. Under laboratory conditions, deoxynivalenol (DON) concentrations of winter wheat grain were analysed by accepted methods (SCHWADORF & MÜLLER 1991). Furthermore, several grain quality traits of winter wheat were determined using standard methods (KLÜVER 1994).

## Methodological problems in field investigations on *Fusarium graminearum* infection of wheat

### Methodische Probleme bei Feldversuchen zum Befall von Weizen mit *Fusarium graminearum*

Cuilin Yi\*, H.-P. KAUL, E. KÜBLER, W. AUFHAMMER

University of Hohenheim, Institute for Crop Production and Grassland Research, Fruwirthstr. 23, D-70599 Stuttgart, Germany

\* corresponding author

Received 17 April 2000; accepted 10 July 2000

### Summary

Cereal head blight, caused by *Fusarium graminearum*, is a major disease problem. The pathogen is able to spread spores across large distances within crops. For plot experiments in the field with artificial inoculation, it has to be considered whether individual treatments can be efficiently separated from each other by isolation strips. In a 2-years field experiment with wheat performed in south Germany, strips of different width (2, 4, 8 m) were planted with tall-growing winter rape cultivars. They separated wheat plots artificially inoculated with *F. graminearum*-infected oat grains from non-inoculated wheat test plots. Disease incidence (% infected ears) and severity (infected spikelets per infected ear) were investigated on the basis of plot scores and detailed observations of individual ears. The positive correlation between disease incidence and severity was only moderate. Thus, both traits supply supplemental information about the infection level. The assessment of cereal *Fusarium* infections can be seriously obstructed by similar visual symptoms due to contemporary infections with other diseases. The isolation strips did significantly reduce the infection on test plots. The isolation effect improved slightly with increasing strip width, but infection was not completely eliminated even with 8 m wide strips. In conclusion, adequate field experiments need a properly randomized design and a sufficient number of replicates.

**Key words:** *Fusarium graminearum*; wheat; investigation method; isolation strips; spikelet infection

### Zusammenfassung

Weißährigkeit aufgrund von Befall mit *Fusarium graminearum* ist eine bedeutende Getreidekrankheit. Der Erreger vermag seine Sporen über große Entfernungen innerhalb von Feldbeständen zu verbreiten. Bei Parzellenversuchen mit künstlicher Inokulation stellt sich die Frage, ob unterschiedliche Behandlungen effektiv durch Isolationsstreifen voneinander getrennt werden können. In einem zweijährig durchgeführten Feldversuch mit Weizen unter süddeutschen Klimabedingungen wurden Isolationsstreifen unterschiedlicher Breite (2, 4, 8 m) mit hochwachsenden Winterrapsorten besät. Diese trennten Weizenparzellen, die mit *Fusarium graminearum* infizierten Haferkörnern inokuliert wurden, von nicht-inokulierten Weizen-Testparzellen. Die Befallshäufigkeit (% befallene Ähren) und die Befallsstärke (befallene Ährchen je befallene Ähre) wurden auf der Grundlage von Parzellenbonituren und von detaillierten Einzelährenbeobachtungen erhoben. Die Korrelationen zwischen Befallshäufigkeit und -stärke waren verhältnismäßig schwach, so dass beide Merkmale einander ergänzende Informationen zum Befallsausmaß liefern. Die Bonitur von Weißährigkeit infolge *Fusarium*-Befall kann durch

ähnliche Symptome gleichzeitig auftretender anderer Krankheiten beeinträchtigt werden. Die Isolationsstreifen reduzierten den Befall auf den Testparzellen nachweislich. Der Isolationseffekt nahm mit ansteigender Streifenbreite zu, aber selbst 8 m breite Isolationsstreifen konnten den Befall nicht vollständig ausschalten. Infolgedessen erfordern sachgerechte Feldversuche eine konsequente Randomisierung der Versuchsglieder sowie eine ausreichende Zahl von Feldwiederholungen.

**Stichwörter:** *Fusarium graminearum*; Weizen; Untersuchungsmethode; Isolationsstreifen; Ährchenbefall

## 1 Introduction

Head blight of cereals, caused by *Fusarium* spp., has become a major disease problem world-wide. In China, in the USA and also in some European countries and in south Germany, *F. graminearum* is the dominating *Fusarium* species which can cause severe yield losses (SUTTON 1982; WANG 1996). Additionally, the pathogen produces toxic metabolites contaminating human food and animal feedstuff. The most prominent toxin is deoxynivalenol (DON).

The infection can originate from conidia or from ascospores produced in perithecia on residues of preceding crops such as maize and wheat. It is favoured by periods of high air humidity (> 92 % relative humidity, COOK 1981) and high temperatures (18–30 °C; SUTTON 1982; OBST 1988; BECK et al. 1993; PARRY et al. 1995; WEINERT and WOLF 1995; SCHWEYDA 1996). An infection may occur at any time from ear emergence to maturity, but the disease is most severe after infections in the flowering period (ANDERSON 1948; DIEHL 1984; MIELKE 1988; GANG 1997).

Rainfall and wind are involved in the dispersal of *Fusarium* spores. STRAUSBAUGH and MALOY (1986) found that much more ears were infected with *F. graminearum* and other *Fusarium* spp. in wheat crops receiving overhead irrigation (89 %) compared with crops which did not receive irrigation (0 %). Obviously, rain played an important role in the dispersal of *Fusarium* inoculum, in the infection process, or in both. JENKINSON and PARRY (1994) spotted simulated raindrops on sporodochia of *F. culmorum*. They observed a splash dispersal of conidia at a maximum height of 60 cm above the sporodochia and at a maximum horizontal distance of  $\approx$  100 cm. The conidia are hydrophilous and presumably spread mainly by rain splashes (DIEHL 1984). With *F. graminearum*, however, ascospores are much more important for a primary infection. WANG (1996) showed that the airborne *Fusarium* spores in cereal crops were mainly ascospores and that their incidence was closely related to rainfall events. The number of spores usually peaked on days with rain and 1–2 days afterwards. He found that ascospores were released actively only 1–2 cm above the soil surface but they can be transported across long distances by wind. Conidia, on the other hand, were only released and spread under conditions of wind and rainfall. Other authors assume from field observations of disease occurrence that spores of *F. graminearum* and *F. culmorum* can be disseminated by wind up to 50 m in height and 30 m in horizontal distance from the source (BECK et al. 1997; FERNANDO et al. 1997; LEPSCHY et al. 1997).

MESTERHAZY (1996a, b) found close correlations between visual head symptoms of *Fusarium* incidence on wheat and percentage infected kernels with  $r = 0.87$  up to 0.99. The correlations of visual symptoms and toxin (DON) concentrations or yield loss were similar with  $r = 0.89$  up to 0.96 or  $r = 0.80$  up to 0.97, respectively. Disease incidence on individual ears and disease severity, assessed by the number of infected spikelets per ear, reflect different, only partly overlapping processes during crop infection (WILCOXON et al. 1992). HUI et al. (1997) confirm comparatively close correlations between disease incidence and DON concentrations, but the relationship between disease incidence and disease severity was less pronounced. They revealed by single kernel analysis that DON can also be found in spikelets other than those showing symptoms. HERMANN et al. (1998) indicated substantially lower correlations between visual scorings on a whole plot basis (1–9) and percentage infected kernels ( $r = 0.45$ ) or DON concentration ( $r = 0.28$ ).

The aim of the present study was to answer the following questions:

- How strong are the correlations between the results of different methods to investigate disease incidence and disease severity of *F. graminearum* in plot experiments? Is it necessary to observe both traits separately?

- Is it possible to limit the dispersal of *F. graminearum* from inoculated plots to non-inoculated test plots of wheat by isolation strips of tall-growing winter rape crops?

2 Materials and methods

2.1 Experimental design

A factorial field experiment was conducted at the experimental station Ihinger Hof of Hohenheim University in 1997/98 with winter wheat and 1998/99 with spring wheat, arranged in a complete randomized block design with four replicates (Fig. 1, Table 1). The treatments were 2 m, 4 m and 8 m wide isolation strips of tall-growing winter rape crops separating non-inoculated test plots of wheat from inoculated wheat plots. Winter rape was chosen for the isolation purpose because tall-growing genotypes are available and rape crops reach their maximum height earlier than wheat. The orientation of inoculated and test plots was in parallel with the main wind direction, i. e. from west to east. The wheat crops on inoculated plots were artificially inoculated by *F. graminearum*-infected oat grains. For this purpose, 10 g m<sup>-2</sup> of infected grains were broadcast in the inoculated plots at the beginning of the growing season (Obst 1994). Due to dry weather conditions after inoculation of winter wheat in

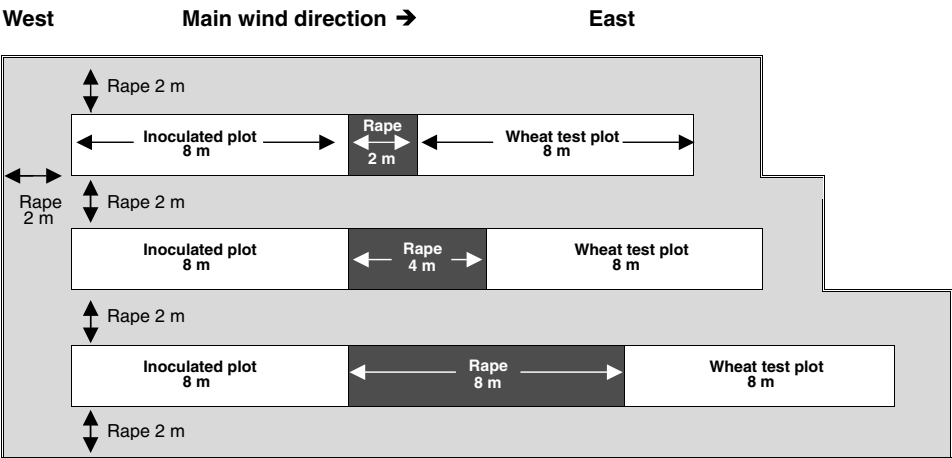


Fig. 1. Experimental design (one replicate).  
Abb. 1. Feldversuchsanlage (ein Wiederholungs-Block).

Table 1. Experimental details  
Tab. 1. Details der Versuchsanlage

Experimental period	1997/98–1998/99	
Location (altitude, temp., rainfall)	Ihinger Hof (480 m a. s. l., 8 °C, 690 mm)	
Inoculated and test crop (cultivar)	1998: Winter wheat (cv. ‘Flair’), sown 28 Oct. 1997 1999: Spring wheat (cv. ‘Quattro’), sown 26 March 1999	
Isolation-strip crop (cultivar)	1998: Winter rape (cv. ‘Lirajet’), sown 02 Sept. 1997 1999: Winter rape (cv. ‘Express’), sown 26 Aug. 1998	
N-fertilizer (Calcium ammonium nitrate, CAN, 27 % N)	Winter rape Winter wheat Spring wheat	} 40 kg ha <sup>-1</sup> N at beginning of growing season + 60 kg ha <sup>-1</sup> N before stem elongation

1997/98, inoculum was applied a second time at the same amount 1 week later. There were no fungicides applied, neither on wheat plots nor on rape strips.

2.2 Observations

Fifty ears were sampled at random from each plot three times at medium (EC75) and late milk ripeness (EC77) and early dough ripeness (EC83), respectively (Tab. 2). From these samples, both the number of infected ears (disease incidence) and the number of infected spikelets were counted. Finally, the number of infected spikelets per infected ear (disease severity) was calculated. For the calculation of disease severity, the number of infected spikelets is usually related to the number of ears under observation (JAMES and SHIH 1973; WILCOXON et al. 1992), but we chose the number of infected ears as the denominator in order to separate more distinctly the infection of different ears and the disease spread within individual initially infected ears. Although the usual calculation of disease severity from all randomly sampled ears is based on a constant sample size, the inclusion of completely healthy ears is questionable. Additionally, in the spring wheat experiment (1998/99), the infection was scored (1–9, non-linear scale) on a whole plot basis (MIEDANER 1986). Due to an infection with foot rot diseases of winter wheat crops grown in 1997/98, it was not possible to assess the infestation with *Fusarium* by whole plot scores in this year. Presumably, these foot rot diseases were a consequence of the crop rotation, because the crop before the experimental winter wheat (1997/98) had been winter wheat, too.

2.3 Statistical analysis

The plot scores (only in 1998/99), the percentages of infected ears and the numbers of infected spikelets per infected ear were analyzed separately from each experiment and sampling date using the General Linear Model (GLM) of SAS (SAS 1989). At first, the effect of the individual treatments was assessed by comparing the test plots with the respective inoculated plots. Subsequently, a covariance analysis with the three levels of isolation strip width as a factor and the observations from the respective test plots as a covariable was performed. The corrected means, as adjusted for the effect of the covariable, are indicated as results. With significant effects ( $p < 0.05$ ) of the isolation strip width, these adjusted means were separated using a Student's *t*-test.

Table 2. Measured and calculated traits  
Tab. 2. Gemessene und errechnete Merkmale

Plot score (1-9)	Infection %	Description (MIEDANER 1986)
1	0	No infection visible
2	1–5	Bleaching of individual spikelets
3	6–15	Individual bleached spikelets at each ear
4	16–25	Bleaching of adjacent spikelets
5	26–45	50 % bleached spikelets per ear
6	46–65	66 % bleached spikelets per ear
7	66–85	75 % bleached spikelets per ear
8	86–95	Bleached spikelets exceed 75 %
9	96–100	Ear completely bleached
Evaluation of ear and spikelet infection	Fifty ears were sampled at random from each plot at EC75, EC77 and EC83. From these samples, infected ears and spikelets were counted.	
Calculation	Disease incidence = Infected ears (% , based on 50 ears) Disease severity = Infected spikelets per infected ear (number, based on 50 ears)  Infection ratio (%) = $\frac{\text{Infected ears or spikelets of the test plot}}{\text{Infected ears or spikelets of the inoculated plot}}$	

The relationships between different traits measured to characterize the infection of wheat with *F. graminearum* were assessed by correlation analysis. Pearson correlation coefficients were calculated by the procedure CORR from SAS. The analysis was performed separately for years and either with results only from test plots or with results from inoculated and test plots. Assuming different levels of infection on these two types of plots, a significant correlation coefficient might be a result only of this comparatively large difference.

### 3 Results

#### 3.1 Conditions for infection in both experiments

The tall-growing winter rape crops on the isolation-strips developed comparatively fast. At ear emergence of wheat crops, the canopy height of rape was 40 cm (in 1999) to 55 cm (in 1998) above the wheat canopies.

The decade means of temperature and wind speed, as well as the sums of precipitation during the experiments are given in Figure 2. The data suggest that the precipitation conditions were more favourable for infection of *F. graminearum* after inoculation in 1999, while in 1998, the inoculation period was comparatively dry. Moreover, the sum of precipitation from May to August was much higher in 1999. Additionally, the wind was stronger in that period. Differences in temperature between years were less prominent. In agreement with these weather conditions, disease incidence and severity on inoculated plots were stronger with spring wheat in 1999 (Table 3, 4). Nevertheless, the infection level of inoculated winter wheat plots in 1998 leaves no doubt that the inoculation was successful.

#### 3.2 Effects of isolation strips

In the winter wheat experiment, investigated in 1998, only at the first sampling date (EC 75) the differences between inoculated plots and test plots in disease incidence and severity were significant (Table 3). Later during crop development, both traits were still lower on test plots, but in most cases the differences were no longer significant. The levels of disease incidence and severity increased slightly during the observation period. Obviously, there was a considerable variance between inoculated plots. Disease incidence on test plots was between 12 % and 67 % of that on inoculated plots and the infection ratio increased with crop development from EC 75 to EC 83. The infection ratio with reference to disease severity was between 63 % and 75 % at EC 75. It did not apparently increase with time, and the maximum number of infected spikelets per infected ear on test plots was only 2.2. Under the specific conditions of this experiment, trends but no consistent effects of isolation strip width either on disease incidence or on disease severity were observed.

In the spring wheat experiment, investigated in 1999, the infection with *F. graminearum* was generally stronger (Table 4). Already at the first sampling date at EC 75, inoculated plots were scored for head blight at 7–8, they showed a disease incidence of 46–62 % and 2.4–3.5 infected spikelets per infected ear. Again the infection symptoms increased slightly during subsequent crop development. At this higher initial level of infection, the results from test plots were generally below those from inoculated plots. The differences were larger with view to plot scores and disease incidence compared with disease severity. The infection ratios were at a similar level compared with the winter wheat experiment in 1998. They showed no obvious tendency of change with crop development. In most cases, they decreased with increasing isolation strip width. But the reduction of infection due to wider isolation strips, as assessed with three different methods, was significant only at EC 83 with view to the plot scores and to disease incidence.

#### 3.3 Evaluation methods for *Fusarium* infection

The correlations between plot scores and the more detailed measurements of disease incidence and severity, which could only be determined in 1999, were low when restricted only to test plots (Table 5). With inoculated plots included, the correlations improved, but they were mainly due to the differences in infection level between the two types of plot (results not presented). The correlations between



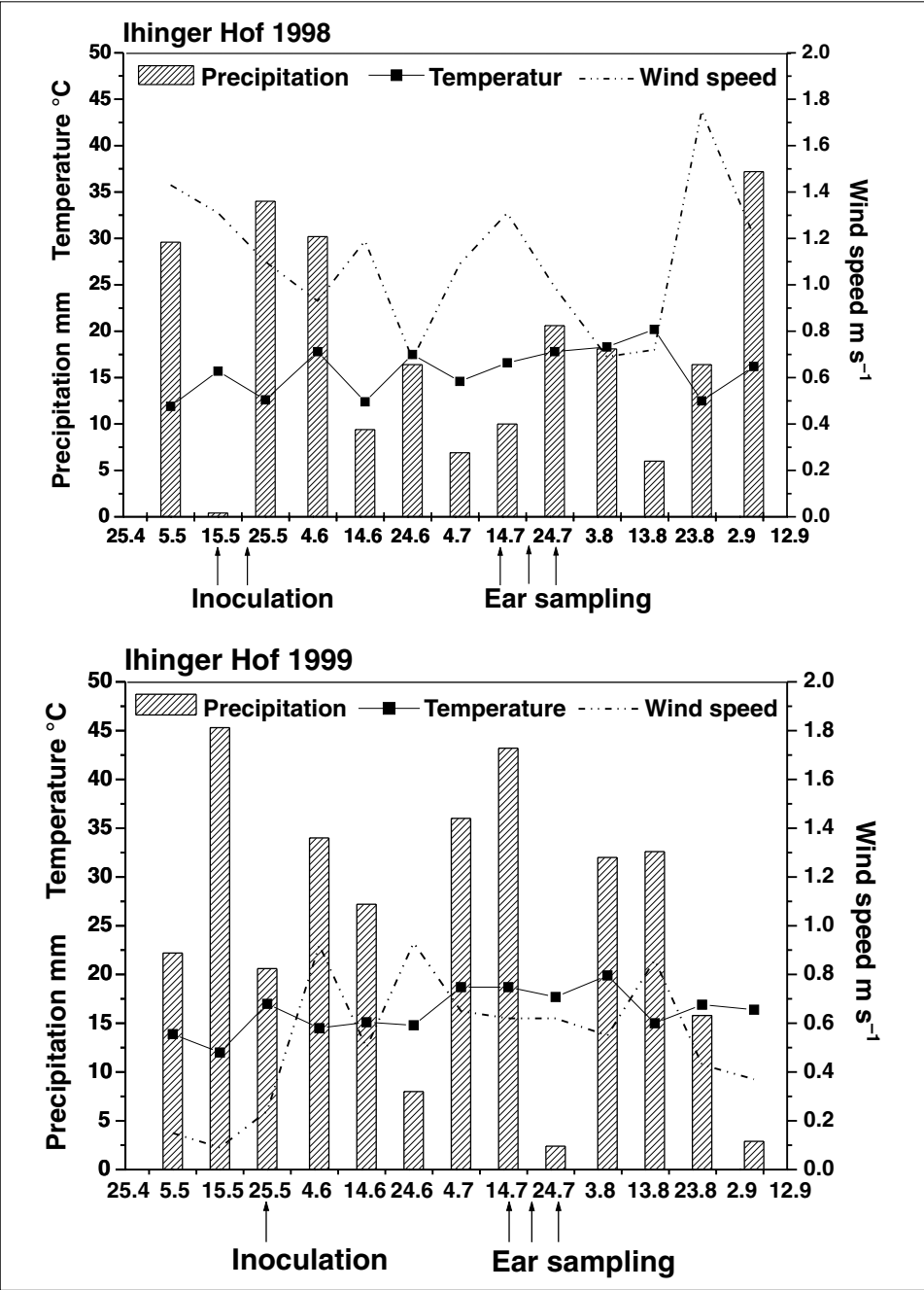


Fig. 2. Decade means of temperature (°C) and wind speed (m s<sup>-1</sup>), sums of precipitation (mm) from May to August and the dates of inoculation as well as the dates of wheat ear sampling.  
Abb. 2. Dekaden-Mittelwerte von Lufttemperatur (°C), Windgeschwindigkeit (m s<sup>-1</sup>) und Niederschlagssummen (mm) von Mai bis August sowie Inokulations- und Probenahmetermine.

Table 3. Disease incidence and disease severity of *F. graminearum* on winter wheat in 1998 as well as the infection ratio (%) dependent on isolation strip width at sequential sampling dates (means from test plots with different letters are significantly different with  $p < 0.05$ )

Tab. 3. Befallshäufigkeit und Befallsstärke von *F. graminearum* an Winterweizen 1998 sowie relativer Befall (%) in Abhängigkeit von der Breite der Isolationsstreifen und vom Entwicklungsstadium (Mittelwerte für „test plots“ mit unterschiedlichen Buchstaben unterscheiden sich nachweisbar,  $p < 0,05$ )

Growth Stage		Disease incidence Infected ears percentage (%)			Disease severity Infected spikelets per infected ear		
		2 m	4 m	8 m	2 m	4 m	8 m
EC75	Inoculated plot	30.0	18.0	43.0	1.6	1.4	1.9
	Test plot	9.0a	4.0a	5.0a	1.2a	1.0a	1.2a
	Inoculated plot > test plot	*	**	***	n. s.	**	**
	Infection ratio (%)	30.0	22.2	11.6	75.0	71.4	63.2
EC77	Inoculated plot	28.0	26.5	33.0	2.8	1.8	2.1
	Test plot	5.5a	4.2a	15.2a	2.2a	1.4a	1.6a
	Inoculated plot > test plot	*	**	*	n. s.	n. s.	n. s.
	Infection ratio (%)	19.6	15.8	46.1	78.6	77.8	76.2
EC83	Inoculated plot	38.5	42.0	31.0	1.9	2.0	2.1
	Test plot	25.8a	23.9a	15.8a	1.4ab	1.7a	1.1b
	Inoculated plot > test plot	n. s.	n. s.	**	n. s.	n. s.	n. s.
	Infection ratio (%)	67.0	56.9	51.0	73.7	85.0	52.4

\* Difference between inoculated and test plot significant at  $P = 0.05$ ; \*\* significant at  $P = 0.01$ ; \*\*\* significant at  $P = 0.001$   
n. s. (not significant)

disease incidence and severity were considerable only in 1999, when the infection level was comparatively high. Even then, they accounted for less than 50 % of the observed variability. It has to be considered that disease severity had been calculated with reference to the number of infected ears only, thus referring mainly to the process of disease spread within initially infected ears.

4 Discussion

Plot scores of head blight symptoms are easy to obtain, but their correlation with a more detailed investigation of disease incidence, based on the examination of randomly sampled individual ears, is often not significant (MESTERHAZY 1996; HERMANN et al. 1998). The inoculation method – either similar to nature by infected oat grains or completely artificial by spraying spore suspensions on the ears – plays an important role for the infection level. The present results from only 1 year (1999) showed an apparently close relationship, but this was essentially caused by the large difference in infection level between inoculated and test plots. The scoring for *Fusarium* infection of crops infected with other diseases, e. g., foot rot, causing symptoms similar to *Fusarium* head blight, can even be impossible as found during the present study with winter wheat in 1998.

The relationship between disease incidence, i. e., percentage infected ears, and disease severity, i. e., the number of infected spikelets per infected ear, turned out to be strong with a comparatively high level of infection in 1999, when disease incidence on inoculated plots reached close to 100 % and disease severity went up to nine infected spikelets per infected ear (Fig. 3). In this year, the correlation was not mainly caused by the different infection levels of inoculated and test plots, but it was also found with reference only to the test plots. The correlation coefficients indicated only a moderate association. Thus, the relations between disease incidence and severity do not encourage the use of only one trait in general to characterize the infection level of different crops (HUI et al. 1997). Furthermore, it has to be realized that predominantly at moderate infection levels the correlations between disease incidence or severity, on one hand, and toxin concentration in grains or yield reduction, on the other hand, are often found to be low,

Table 4. Plot score (1–9), disease incidence and disease severity of *Fusarium graminearum* on spring wheat in 1999 as well as the infection ratio (%) dependent on isolation strip width at sequential sampling dates (means from test plots with different letters are significantly different with  $p < 0.05$ )

Tab. 4. Befallsbonituren (1–9), Befallshäufigkeit und Befallsstärke von *F. graminearum* an Sommerweizen 1999 sowie relativer Befall (%) in Abhängigkeit von der Breite der Isolationsstreifen und vom Entwicklungsstadium (Mittelwerte für „test plots“ mit unterschiedlichen Buchstaben unterscheiden sich nachweisbar,  $p < 0,05$ )

Growth Stage		Plot score (1–9)			Disease incidence Infected ears percentage (%)			Disease severity Infected spikelets per infected ear		
		2 m	4 m	8 m	2 m	4 m	8 m	2 m	4 m	8 m
EC75	Inoculated plot	7.9	7.6	7.6	48.0	45.5	61.5	2.5	2.4	3.5
	Test plot	4.1a	3.2a	2.6a	8.8b	4.0b	17.1a	1.2a	1.0a	1.4a
	Inoculated plot > Test plot	***	***	***	***	***	***	**	***	*
	Infection ratio (%)	51.9	40.8	34.2	17.7	7.7	29.3	48.0	41.7	40.0
EC77	Inoculated plot	8.5	8.1	8.0	66.0	66.5	78.5	2.7	2.2	4.1
	Test plot	3.7a	2.8a	2.5a	12.4a	8.4a	6.7a	1.4a	1.8a	1.6a
	Inoculated plot > Test plot	***	***	***	***	***	***	**	n. s.	**
	Infection ratio (%)	41.2	35.8	32.5	18.9	12.8	8.3	51.9	81.8	39.0
EC83	Inoculated plot	8.5	8.3	8.6	69.5	76.0	71.0	4.9	5.4	4.8
	Test plot	3.4a	3.3a	2.5b	26.5a	24.2ab	13.4b	3.6a	3.3a	1.8a
	Inoculated plot > Test plot	***	***	***	***	***	***	n. s.	**	***
	Infection ratio (%)	40.0	39.7	29.1	41.7	27.0	20.4	73.5	61.1	37.5

\* Difference between inoculated and test plot significant at  $P = 0.05$ ; \*\* significant at  $P = 0.01$ ; \*\*\* significant at  $P = 0.001$   
n. s. (not significant)

Table 5. Correlation coefficients for different traits measured to characterize the infection with *Fusarium graminearum*

Tab. 5. Korrelationskoeffizienten für verschiedene Merkmale des Befalls mit *F. graminearum*

Traits		Infected ears percentage (%)		Infected spikelets per infected ear	
		1998	1999	1998	1999
Plot score (1–9)	Only test plots (n = 36)	n. d.	0.07	n. d.	0.32
	Inoculated plots included (n = 72)	n. d.	0.92***	n. d.	0.56***
Infected ears (%)	Only test plots (n = 36)			0.10	0.66***
	Inoculated plots included (n = 72)			0.39**	0.68***

\*\* significant at  $P = 0.01$ ; \*\*\* significant at  $P = 0.001$   
n. d. (not determined)

even with detailed investigations of the infection level (SNIJDERS and PERKOWSKI 1990; HERMANN et al. 1998). In general, the experimental basis of the present investigation on the correlations between different traits measured to characterize the infection level was comparatively small.

The present study showed that isolation strips are a suitable measure to limit the spread of *F. graminearum* spores in plot experiments in the field with artificially inoculated plots. Isolation strips of 2 m

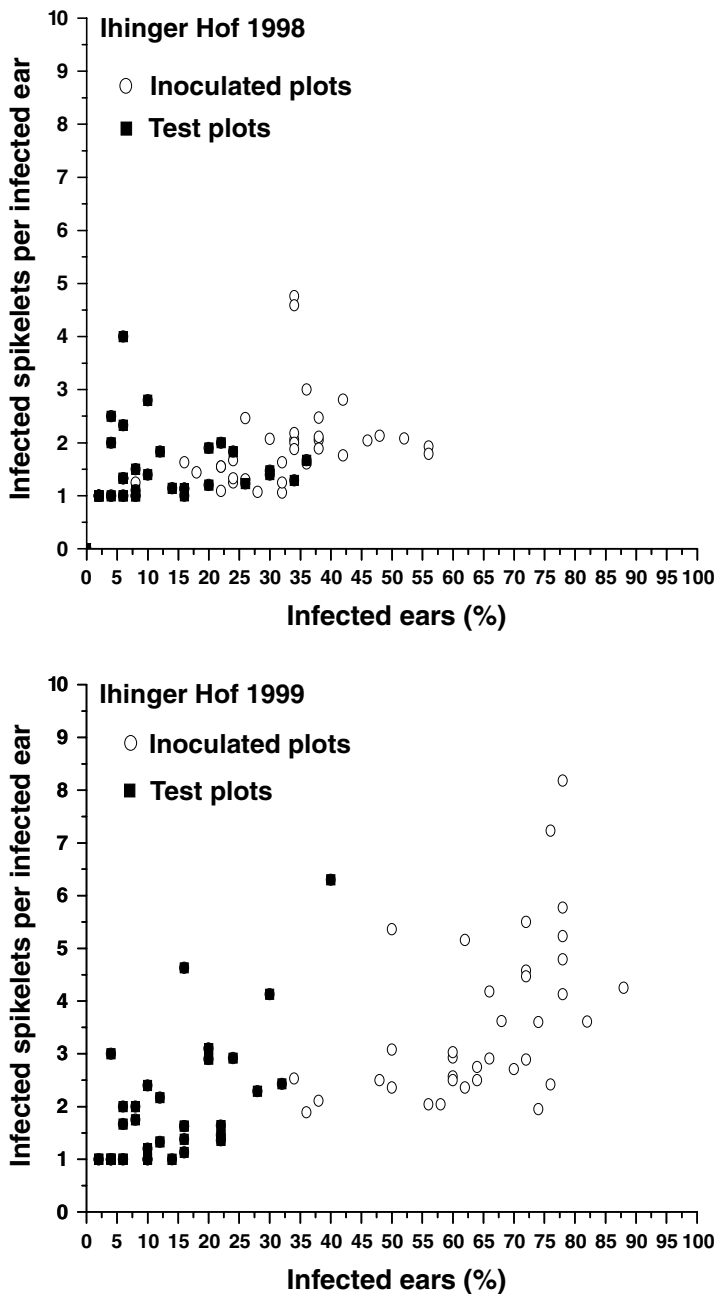


Fig. 3. Relationship between disease severity and disease incidence as influenced by year and plot type.  
Abb. 3. Beziehungen zwischen Befallsstärke und Befallshäufigkeit in Abhängigkeit von Jahr und Parzellentyp.

width of winter rape crops growing about 50 cm higher than the wheat crops reduced disease incidence on test plots by more than 50 % in most cases. The reduction of disease severity was less pronounced. This can be attributed to the fact that disease severity is only partly an effect of initial infection but it is also the result of the further development of the pathogen on the initially infected ears.

A further increase in isolation strip width above 2 m did improve the isolation effect, but the differences between isolation strip widths were comparatively small. It has to be emphasized that the spread of inoculum from plots inoculated with infected oat grains on the soil cannot be reduced to a negligible level even by isolation-strips of 8 m width. The assumption of JENKINSON and PARRY (1994), that conidia can pass distances of only about 1 m within field crops, is not supported by the present results. According to BECK et al. (1997), it seems much more pertinent that distances up to 30 m from the source can be passed, at least without any isolation strips inserted. Up to now, no other experimental data are available to recommend more suitable crops or cultivars for the isolation purpose. Species like hemp or sunflower grow much taller than winter rape, but because they develop too slow in spring compared with wheat crops, at least under our climatic conditions, they are not suitable for segregation.

The assessment of cereal *Fusarium* infections, especially scorings on a whole plot basis, can be seriously obstructed by similar visual symptoms due to contemporary infections with other diseases. Therefore, experimental fields should be free from soil-borne cereal diseases like foot rot. Otherwise, these diseases have to be controlled by fungicides. But one can never be sure whether specific active substances in interaction with environmental conditions develop any not intended side-effects against *Fusarium* spp. And, thus, interfere with the experimental treatments under study. And it must be realized that a contemporary disease infection with interactions between different pathogens is reality under applied conditions.

Contrasting to artificial inoculations by spraying spore suspensions on the ears, the inoculation of wheat with *F. graminearum* by infected oat grains during early stages of crop development mimics the natural infection conditions. The infection starts from residues of preceding crops on the soil surface and it is influenced by characteristics of the crop canopy and wind effects. The wind dispersal of ascospores, however, causes severe problems in field plot experiments due to not intended contamination of non-inoculated plots. Of course, spray inoculation allows for a much better control of the infection, but this method excludes the natural infection process starting at the soil surface.

Winter rape strips of 8 m width did not prevent the dispersal of *Fusarium* spores from inoculated plots to non-inoculated test plots of wheat completely. During early stages of ripening (EC 75), the infection ratio of test plots was only 10–20 % compared with the inoculated plots, but it increased up to about 50 % at EC 83. Even with 8 m wide isolation strips, the interference of plots with different levels of infection within one field experiment cannot be excluded. Under these conditions, the statistical significance of effects on the infection level due to factors of crop husbandry can only be expected from experiments with a properly randomized design and a sufficient number of replicates.

## Literature

- ANDERSON, A. L.: The development of *Gibberella zeae* head blight of wheat. – *Phytopathology* **38**, 595–611, 1948.
- BECK, R., J. LEPSCHY, A. OBST: Gefahr aus der Maisstoppel. – *DLG-Mitteilungen* **5/97**, 34–38, 1997.
- BECK, R., A. SÜSS, J. LEPSCHY: *Fusarien* verantwortlich für Bierfehler? – *Pfl.Schutz-Praxis* **2/93**, 26–29, 1993.
- COOK, R. J.: *Fusarium* diseases of wheat and other small grains in North America. – In: Nelson, P. E., T. A. Toussoun, R. J. Cook (eds.): *Fusarium-diseases, biology, and taxonomy*, pp. 39–52. – Pennsylvania State University Press, University Park, PA, 1981.
- DIEHL, T.: Weizenfusariosen – Zur Symptomentwicklung und Schadensanalyse bei Blatt- und Ährenbefall. – PhD Thesis, Univ. Göttingen, Germany, 1984.
- FERNANDO, W. G. D., T. C. PAULITZ, W. L. SEAMAN, P. DUTILLEUL, J. D. MILLER: Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. – *Phytopathology* **87**, 414–421, 1997.

- GANG, G.: Genetische, umweltbedingte und physiologische Einflüsse auf die Resistenz bzw. Aggressivität im Pathosystem Roggen *Fusarium culmorum* (W. G. Sm) Sacc. – PhD Thesis, Univ. Hohenheim, Stuttgart, Germany, 1997.
- HERMANN, W., E. KÜBLER, W. AUFHAMMER: Ährenbefall mit *Fusarium* und Toxingehalt im Korngut bei verschiedenen Wintergetreidearten. – Pflanzenbauwissenschaften 2, 97–107, 1998.
- HUI, Y., C. K. EVANS, E. K. KOLACZKOWSKI, M. R. DILL, C. J. MIROCHA, Y. HUI: Chemistry, physiology and role of deoxynivalenol in pathogenicity. – Bulletin of the Institute for Comprehensive Agricultural Sciences, Kinki University. No. 5, 1–11, 1997.
- JAMES, W. C., C. S. SHIH: Relationship between incidence and severity of powdery mildew and leaf rust on winter wheat. – Phytopathology 63, 183–187, 1973.
- JENKINSON, P., D. W. PARRY: Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. – Mycol. Res. 98, 506–510, 1994.
- LEPSCHY, J., R. BECK: Vorkommen und Bedeutung von Fusarien und ihren Toxinen im Getreide. – Bodenkultur und Pflanzenbau, Sondernummer 1, 1997.
- MESTERHAZY, A.: Breeding for resistance to fusarium head blight of wheat. – In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: global status and future prospects. – International Maize and Wheat Improvement Center, pp. 79–85, 1996a.
- MESTERHAZY, A.: Fungicide control of fusarium scab and impact on toxin contamination. – In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: global status and future prospects. – International Maize and Wheat Improvement Center, pp. 120–124, 1996b.
- MIEDANER, T.: Entwicklung von Methoden zur Bestimmung der Fusarienresistenz in frühen Wachstumsstadien des Weizens. – PhD Thesis, Univ. Hohenheim, Stuttgart, Germany, 1986.
- MIELKE, H.: Untersuchungen über *Fusarium culmorum* (W. G. Sm) Sacc. als Fuss- und Blattkrankheitserreger beim Weizen. – Mitt. Biol. BundAnst. Ld.- u. Forstw. H. 238, 1–101, 1988.
- OBST, A.: Wie man Ährenfusariosen vermeidet. – DLG-Mitteilungen 50, 470–471, 1988.
- PARRY, D. W., P. JENKINSON, L. MCLEOD: *Fusarium* ear blight (scab) in small grain cereals – a review. – Pl. Path. 44, 207–238, 1995.
- SCHWEYDA, H. J.: Pathogene und fakultativ pathogene samenbürtige Pilze auf Saatgetreide aus Baden-Württemberg: Biologie – Befallssituation – Konsequenzen. – PhD Thesis, Univ. Karlsruhe, Germany, 1996.
- SNIJEDERS, C. H. A., J. PERKOWSKI: Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. – Phytopathology 80, 566–570, 1990.
- STRAUSBAUGH, C. A., O. M. MALOY: *Fusarium* scab of irrigated wheat in Central Washington. – Pl. Dis. 70, 1104–1106, 1986.
- SUTTON J. C.: Epidemiology of wheat head blight and maize ear rot caused by *F. graminearum*. – Can. J. Pl. Pathol. 4, 195–209, 1982.
- WANG, Y. Z.: Epidemiology and management of wheat scab in China. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: global status and future prospects. – International Maize and Wheat Improvement Center, pp. 97–105, 1996.
- WEINERT, J., G. A. WOLF: Gegen Ährenfusarien helfen nur resistente Sorten. – Pfl.Schutz-Praxis 2/95, 30–32, 1995.
- WILCOXSON, R. D., R. H. BUSCH, E. A. OZMON: *Fusarium* head blight resistance in spring wheat cultivars. – Pl. Dis. 76, 658–661, 1992.

## Populations of *Fusarium graminearum* on crop residues as affected by incorporation depth, nitrogen and fungicide application

Cuilin Yi, H.-P. Kaul, E. Kübler, W. Aufhammer

Journal of Plant Diseases and Protection

### Summary

*Fusarium* head blight of wheat, caused by *Fusarium graminearum*, is an important disease world-wide. Its increasing incidence has been attributed to reduced tillage and more crop residues on the soil surface. Greenhouse tests were conducted to determine how crop residues and disease control measures influence the population level of *F. graminearum* on crop residues. Experimental factors in three similar experiments were (i) residue type (wheat or maize) (ii) incorporation depth (5, 10, 15 cm) and (iii) soil amendment (calcium ammonium nitrate application or nitrolime application), tebuconazole treated residues, or an untreated control. With both residue types, deeper residue incorporation slightly reduced the decomposition process as well as the population level of *F. graminearum*. The application of nitrolime did not affect the decomposition process but reduced the population level of *F. graminearum*. By contrast, fertilization with calcium ammonium nitrate sometimes reduced residue decomposition but enhanced the population level of *F. graminearum*. Soaking the residues in a fungicide preparation impaired the decomposition process substantially and eliminated *F. graminearum* on the residues completely. The dependence between residue decomposition and Colony Forming Units was comparatively close, but decomposition and macroconidia density were weakly associated. It is concluded that nitrolime can help to reduce the populations of *F. graminearum* on the residues without detrimental effects on the decomposition process. Burying *F. graminearum* infested crop residues deeper in the soil can effectively reduce *F. graminearum* populations, but as decomposition is slowed down, the pathogen may survive for longer periods in the soil.

**Key words:** Crop residue, decomposition, *F. graminearum*, macroconidia density, Colony Forming Units (CFU)

## 1 Introduction

*Fusarium* head blight of wheat, caused by *Gibberella zeae* (*Fusarium graminearum* Schwabe), is an important disease world-wide. It causes serious yield losses and the contamination of grain with toxic metabolites (FERNANDEZ and FERNANDES 1990; MILLER 1994). The main source of infection are ascospores, formed on crop residues, and conidia (SUTTON 1982). Temperature and water in the soil environment greatly affect sporulation of *F. graminearum* from residues (WANG 1996; GRACIA-GARZA and FRAVEL 1998). The most suitable temperature for producing conidia and ascospores is 15-20°C and the most suitable soil moisture content is 70-80% of field capacity (WANG 1996).

*Fusarium* species attacking small grains survive mainly on crop residues (MCMULLEN and STACK 1983; STURZ and BERNIER 1987). They can survive on crop residues in the soil for more than 6 years as mycelium, ascospores, conidia or chlamydospores (NYVALL 1970; SUTTON 1982; WIESE 1987; REIS 1990; HAWARE et al. 1996). The inoculum level is reduced as stubble decomposes, but even under conditions favourable for decomposition the pathogen can survive for at least a year (SUTTON 1982).

The increased incidence of *Fusarium* head blight world-wide has been attributed to reduced tillage and prolonged residue retention on the soil surface (STURZ and JOHNSTON 1985; SUMMERELL et al. 1990; SALAS 1991; DILL-MACKY 1996; WANG 1996; MILLER et al. 1998; DILL-MACKY and JONES 2000). Crop residue placement, which is dictated by tillage practice, has a significant influence on crop residue decomposition (BROWN and DICKEY 1970; DOUGLAS et al. 1980; DORAN 1987; HOLLAND and COLEMAN 1987). HUBBARD and JORDAN (1996) observed a faster residue decomposition with residues incorporated in the soil than with surface-placed residues, while *Fusarium* head blight incidence and severity were found to be lower in moldboard ploughed plots than in either chisel ploughed or no-till plots (MILLER et al. 1998; DILL-MACKY and JONES 2000).

High soil nitrogen concentrations are favourable for the fast decomposition of crop residues. However, high soil nitrogen can also promote *Fusarium* head blight in wheat (TEICH 1989; MARTIN et al. 1991; AUFHAMMER et al. 2000). The type of nitrogen added is also influential. Thus, the incidence and severity of *Fusarium* root-rot and foot-rot diseases of winter wheat was higher after fertilization with ammonium (NH<sub>4</sub>) compared with nitrate (NO<sub>3</sub>) application, and wheat fertilized with urea (CO(NH<sub>2</sub>)<sub>2</sub>) showed fewer *Fusarium* head blight symptoms than when fertilized with ammonium nitrate (HUBER and WATSON 1965; SMILEY et al. 1972; TEICH 1987; MARTIN et al. 1991).

Fungicides containing tebuconazole have shown efficacy against *Fusarium* head blight and yields were increased by 35% upon application of the fungicide Folicur (a.i.= tebuconazole) (FEHRMANN and DIEHL 1989; JORDAN and HUTCHEON 1989). However, the fungicide effectiveness depends on weather conditions and timing of



application in relation to infection date (OBST et al. 1992). Fungicide application may even increase the mycotoxin concentration of grain (HERMANN et al. 1999). Based on a greenhouse experiment with wheat straw, MOLLENHAUER et al. (1999) concluded that fungicides had no effect on the rate of straw decomposition.

Based on the knowledge outlined above, three main points shall be investigated:

- Is there a close relationship between the decomposition of crop residues and the population level of *F. graminearum* on the residues?
- What are the direct effects of a fungicide or the indirect effects of incorporation depth and additional nitrogen supply on the decomposition of crop residues and the population level of *F. graminearum* on the residues?
- Is it necessary to assess the *F. graminearum* population level on crop residues by investigating Colony Forming Units and macroconidia density separately? Can we assume a strong correlation between the results of these two methods to assess *F. graminearum* population level on crop residues?

## 2 Materials and Methods

### 2.1 Cropping sequence in the field and artificial inoculation

Crop rotations were established at the experimental station “Ithinger Hof” of Hohenheim University at field site A in 1997-99 and field site B in 1998-99 (Table 1). Maize or spring wheat had been planted as first crops in the rotation in spring 1997, (field site A) or 1998 (field site B). Maize and spring wheat crops were inocu-

**Table 1.** Cropping sequence and timing of residue sampling for greenhouse tests

Field site		A		B	
Plot		1	2	3	4
Crop rotation	1997	Maize*	Spring wheat*	pre-experimental period	
	1998	Winter wheat	Winter wheat	Maize* <sup>1)</sup>	Spring wheat* <sup>1)</sup>
	1999	Maize <sup>2)</sup>	Spring wheat <sup>2)</sup>	Winter wheat	Winter wheat

\* Maize and spring wheat were inoculated with *F. graminearum* infected oat grain.

<sup>1)</sup> Shortly after harvest, crop residues for greenhouse test I (11/98-02/99) were sampled in November 1998 from plots which had been inoculated in May 1998 and again in April 1999 from the soil surface under the growing winter wheat crops (greenhouse test II, 04/99-07/99).

<sup>2)</sup> Crop residues for greenhouse test III (11/99-02/00) were sampled in November 1999 from plots which had been inoculated in May 1997.

lated with *F. graminearum* infected oat grains by broadcasting 10 g m<sup>-2</sup> of infected grain on the soil surface on 14 May and 25 May in 1997 and 1998, respectively. After maize and spring wheat, winter wheat was sown in autumn 1997 (field site A)

or 1998 (field site B). respectively. At field site A, the winter wheat crop was followed by either maize or spring wheat in 1999.

## 2.2 Sampling of residues for greenhouse tests

Crop residues (total 9 kg) were randomly sampled once from field site A and twice from field site B (Table 1). Samples were collected in autumn 1998 from field site B, shortly after harvest of the inoculated spring wheat and maize crops (for greenhouse test I, 11/98-02/99), and again from the same plots in spring 1999 (for greenhouse test II, 04/99-07/99). From field site A, crop residues were sampled once in autumn 1999, shortly after harvest of spring wheat and maize crops from the plots which had been inoculated about 2.5 years before (for greenhouse test III, 11/99-02/00). The different samplings were expected to provide crop residues with different levels of *F. graminearum* infestation. All samples (stems including internodes and nodes) of maize and spring wheat were immediately cut to pieces of 2 cm length, dried at 28°C for 96 hours, and used for greenhouse tests.

The factorial design of the greenhouse tests is shown in Table 2. Each 5 g of residue dry matter was placed inside 12 x 18 cm fiberglass-mesh (1 mm<sup>2</sup> meshsize) bags and incorporated with 3600 g of a standard soil mixture in pots (20 cm diameter × 21 cm high) at 5, 10 and 15 cm depth, simulating different burying depths in the field. A surface placement was omitted because contact between soil and the residues wrapped in the bags seemed to be marginal when positioned on the soil surface. Characteristics of the soil and the residues are shown in Table 3. Four treatments were applied to the residues in pots, *i.e.* T1: control (only soil plus straw of maize or wheat in fiberglass-mesh bags); T2: addition of calcium ammonium nitrate (CAN) equivalent to 200 kg N ha<sup>-1</sup>; T3: addition of nitrolime equivalent to 200 kg N ha<sup>-1</sup>; T4: straw treatment with the fungicide Folicur (a.i. tebuconazole, 1.25 g tebuconazole L<sup>-1</sup> water). The fertilizers CAN and nitrolime were ground to a powder and broadcasted on the soil surface of each pot, simulating the agronomic field practice. The ground fertilizers were dispersed over the soil by the water added regularly on the pots to maintain the soil water content at 60% of field capacity. For the fungicide treatment (T4), the residues were soaked in a fungicide preparation for 60 minutes. They were then air-dried again before buried in the pots. This treatment was designed to ensure the residues were in intimate and sufficient contact with the fungicide. Temperatures during the experiments in a greenhouse were 25°C/18°C (day/night). After 30, 60 and 90 days the residues were recovered from the bags and the residual material was analyzed further. Each experimental unit was replicated 8 times per sampling date.

**Table 2.** Factorial design for greenhouse tests

Type of residue	Maize, cv. Helix Spring wheat, cv. Hanno
Treatment (T)	T1: control (only soil plus straw) T2: 0.64 g calcium ammonium nitrate (CAN, 27% N) kg <sup>-1</sup> soil (equivalent to 200 kg N ha <sup>-1</sup> ) T3: 0.86 g nitrolime (20.5% N) kg <sup>-1</sup> soil (equivalent to 200 kg N ha <sup>-1</sup> ) T4: Fungicide treatment (Folicur, a.i. tebuconazole) at 1.25 g tebuconazole L <sup>-1</sup> water
Incorporation depth	5, 10 or 15 cm

## 2.3 Investigation methods

### Residue decomposition

The residue containing bags recovered from the pots at each sampling date were brushed to remove loose soil and dried at 28°C for 72 h. Subsequently the dry weight of the remaining residue per bag was determined. The weight difference between the initial 5 g of residue and that remaining is indicated as the decomposition of residue (g).

### Colony Forming Units (CFU)

The CFU is defined as the number of colonies developing on a suitable agar medium on the basis of 1 g dry matter of the recovered residues (CFU g<sup>-1</sup>). The determination of CFU from residue in greenhouse tests I and II was preceded by surface sterilization of the recovered residue pieces to restrict the analysis to fungi growing within the residue tissue. Surface sterilization was necessary in these experiments because too many colonies developed from non-sterilized residues, making counting difficult. Surface sterilization was performed by immersing the residue pieces in ethanol (70%) for 15 s, followed by immersion in NaOCl (1%) for 15 s. Residues were then rinsed twice with sterile distilled water. The original residues, as sampled from field sites, as well as the samples from greenhouse test III were not surface sterilized. An initial test with sterilized samples had shown that nearly no colonies grew from the residues presumably due to a low infestation level. Therefore, these residues were only rinsed with sterile distilled water once. Because of the difference in sterilization protocol among the greenhouse tests, a comparison of CFU results by absolute number of colonies was not possible. Finally, 1 g dry matter of either sterilized or non-sterilized residue material was added to 99 ml of a

nutrient solution ( $0.57 \text{ g L}^{-1} \text{ NaH}_2\text{PO}_4$ ,  $4.0 \text{ g L}^{-1} \text{ NaCl}$ ,  $2.6 \text{ g L}^{-1} \text{ Na}_2\text{HPO}_4$ ). This mixture was stirred with a magnetic bar for 20 min. Then the suspension was diluted 1:10, 1 ml was placed in petri dishes (14 cm diameter) and covered with autoclaved Dichloro-Glycerol-Agar medium (DG-18 A) (HOCKING and PITT 1980) at about  $45^\circ\text{C}$  in three replicates. This agar medium promotes growth of mycelium. The plates were incubated at room temperature ( $\sim 25^\circ\text{C}$ ) with normal daylight. The colonies were counted after 6 days. The identity of colonies as *F. graminearum* was determined for random samples by examination of the conidial morphology under the microscope.

### Macroconidia density

The macroconidia density is a measure of the sporulation potential of the fungus. It was determined from a second 1 g sub-sample of remaining residue material per litter-bag which was added to 99 ml of sterile distilled water. This mixture was stirred with a magnetic bar for 20 min. A compound microscope and a hemacytometer were used to count the number of macroconidia in 8 replicates of 2 drops from each suspension.

## 2.4 Statistical analysis

The decomposition of residues (g), the Colony Forming Units ( $\text{CFU g}^{-1}$ ) and the macroconidia density ( $\text{g}^{-1}$ ) were statistically analyzed separately for each experiment and sampling date using the General Linear Model (GLM) of SAS (SAS 1989). For significant effects ( $P < 0.05$ ), Least Significant Differences ( $\text{LSD}_{0.05}$ ) for the separation of means were calculated. The statistical associations between the different measured traits were assessed by correlation analysis, calculating Pearson correlation coefficients by the procedure CORR from SAS (SAS 1989). This analysis was performed separately for experiments and treatments.

## 3 Results

### 3.1 Original field residues prior to greenhouse tests

Before the crop residues were used for greenhouse tests, their chemical characteristics were determined (Table 3), as well as the level of Colony Forming Units (CFU) and the macroconidia density from the original non sterilized residues (Table 4). More CFU always developed from wheat than from maize residues. The CFU levels from residues sampled for greenhouse test I were higher than those from residues for greenhouse tests II and III. Compared with greenhouse test I, the number of colonies from residues of maize and wheat for greenhouse test II had declined during winter by 64% and 78%, respectively. On maize and wheat residues for greenhouse test III, sampled in November 1999, we obtained 91-93% less colonies than from the directly inoculated residues for greenhouse test I. No

macroconidia were found on residues of maize or wheat prior to the greenhouse tests. Likely, temperature before sampling had been too cold and as the residues had been very dry for a long time before sampling, they were not a suitable substrate for macroconidia development.

**Table 3.** Characteristics of the soil and crop residues before use in greenhouse tests

Soil traits		Crop residue traits			
		Greenhouse test I		Greenhouse test II	Greenhouse test III
pH	5.8-6.0	Maize:			
NO <sub>3</sub> -N	15.7 mg kg <sup>-1</sup> soil	N (%)	0.6	0.5	0.8
N <sub>t</sub>	0.2%	C (%)	46.3	35.7	43.3
C <sub>org</sub>	8.4%	<i>Spring wheat:</i>			
Clay	9%				
Sand	25%	N (%)	0.4	0.5	0.5
Field cap.	37% H <sub>2</sub> O (w/w)	C (%)	44.9	26.1	45.4

**Table 4.** Colony Forming Units levels of initial field crop residues prior to greenhouse tests (not surface sterilized, n=24)

	Greenhouse test I		Greenhouse test II		Greenhouse test III	
	Maize	Spring wheat	Maize	Spring wheat	Maize	Spring wheat
Colony Forming Units (CFU g <sup>-1</sup> )	11	108	4	24	1	8
LSD <sub>0.05</sub>	7		4		1	

### 3.2 Residues recovered during the greenhouse tests

The analyses of variance revealed regularly (residue decomposition, CFU levels and macroconidia density; data not shown) significant main effects of the three factors studied. Residue decomposition, CFU and macroconidia density also were substantially affected by the interaction of residue type and treatment. Additionally, the association between incorporation depth and treatment was significant for both fungal population indicators. Consequently, the presentation of results is focused on these two interactions. During all greenhouse tests and in all experimental variants, residue decomposition and both fungal traits increased with incorporation days in greenhouse tests. The differences between individual experimental

**Table 5.** Decomposition of residues, Colony Forming Units and macroconidia density as influenced by type of residue, treatment and sampling date (greenhouse test I)

Residue	T*	Decomposition of residues (g) <sup>+</sup>			Colony Forming Units (CFU g <sup>-1</sup> )			Macroconidia density (× 10 <sup>6</sup> g <sup>-1</sup> )		
		30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
Maize	T1	1.07	1.55	1.99	5	54	68	0.03	2.68	2.59
	T2	1.21	1.72	1.93	6	54	66	0.02	1.09	3.61
	T3	0.84	1.55	2.11	1	19	24	0.00	1.39	1.16
	T4	0.70	0.64	1.11	0	0	0	0.00	0.00	0.00
Spring wheat	T1	0.59	1.23	1.54	14	60	116	0.03	5.12	9.22
	T2	0.59	0.97	1.41	20	65	120	0.09	6.21	14.50
	T3	0.61	1.10	1.61	6	36	49	0.00	3.22	5.89
	T4	0.33	0.44	0.63	0	0	0	0.00	0.00	0.00
LSD <sub>0.05</sub>		0.17	0.16	n. s.	2	3	5	0.04	1.38	2.09

\* T = Treatment (T1 = Control; T2 = CAN application; T3 = Nitroline application; T4 = Fungicide treatment).

<sup>+</sup> Difference between initial 5 g of residue and weight of sampling.

**Table 6.** Decomposition of residues, Colony Forming Units and macroconidia density as influenced by type of residues and treatment after 90 days of greenhouse test (greenhouse test II, III)

Residue	T*	Decomposition of residues (g) <sup>+</sup>		Colony Forming Units (CFU g <sup>-1</sup> )		Macroconidia density (× 10 <sup>6</sup> g <sup>-1</sup> )	
		Test II	Test III	Test II (sterilized)	Test III (not sterilized)	Test II	Test III
Maize	T1	2.07	3.40	30	38	0.68	0.89
	T2	1.74	3.07	35	55	0.75	1.67
	T3	2.05	3.04	18	30	0.39	0.31
	T4	0.86	1.30	0	0	0.00	0.00
Spring Wheat	T1	2.27	2.23	24	29	0.17	0.30
	T2	2.01	1.96	22	38	0.24	0.39
	T3	2.49	2.17	12	16	0.06	0.08
	T4	1.16	1.09	0	0	0.00	0.00
LSD <sub>0.05</sub>		n. s.	0.14	2	4	0.18	0.29

\* T = Treatment (T1 = control; T2 = CAN application; T3 = nitroline application; T4 = Fungicide treatment).

<sup>+</sup> Difference between initial 5 g of residue and weight of sampling.

variants increased also with time. Thus, we present the results for all sampling dates only for greenhouse test I (Tables 5, 7) and restrict the data in tables for the other two greenhouse tests to the results of the last sampling at day 90 (Tables 6, 8).

### 3.3 Residue decomposition

After 30 and 60 days, the decomposition of maize residues was more rapid than that of wheat straw, but after 90 days this difference was evident only for greenhouse test III (Tables 5, 6). The soaking of the residues in a fungicide preparation (T4) strongly decreased the residue decomposition of both crops in each experiment (Tables 5, 6). The effects of the two nitrogen fertilizers CAN and nitrolime on the decomposition process were not consistently different from the control (T1), but after 90 days the decomposition was usually higher with nitrolime (T3) than with CAN (T2) (Tables 5, 6).

**Table 7.** Decomposition of residues, levels of Colony Forming Units and macroconidia density as influenced by burial depth, treatment and sampling date (greenhouse test I)

Burying depth	T*	Decomposition of residues (g) <sup>+</sup>			Colony Forming Units (CFU g <sup>-1</sup> )			Macroconidia density (× 10 <sup>6</sup> g <sup>-1</sup> )		
		30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
5 cm	T1	1.00	1.54	1.87	17	75	113	0.09	6.44	8.52
	T2	1.05	1.48	1.93	25	80	118	0.17	5.83	18.16
	T3	0.96	1.57	2.06	7	42	47	0.00	4.05	6.50
	T4	0.49	0.64	0.98	0	0	0	0.00	0.00	0.00
10 cm	T1	0.74	1.36	1.68	6	50	95	0.00	3.88	5.97
	T2	0.88	1.34	1.68	11	54	94	0.00	2.32	6.82
	T3	0.65	1.27	1.85	4	21	34	0.00	1.52	2.74
	T4	0.49	0.55	0.84	0	0	0	0.00	0.00	0.00
15 cm	T1	0.74	1.28	1.74	5	46	69	0.00	1.38	3.22
	T2	0.77	1.21	1.41	5	45	68	0.00	2.31	2.19
	T3	0.56	1.13	1.68	2	20	30	0.00	1.13	1.33
	T4	0.54	0.38	0.79	0	0	0	0.00	0.00	0.00
LSD <sub>0.05</sub>		n. s.	n. s.	n. s.	3	6	10	0.06	1.89	3.37

\* T = Treatment (T1 = control; T2 = CAN application; T3 = nitrolime application; T4 = Fungicide treatment).

<sup>+</sup> Difference between initial 5 g of residue and weight of sampling.

At the 30 and 60 days sampling dates, decomposition decreased with increasing incorporation depth, but after 90 days this difference had disappeared (Table 7, 8). Except for reduced decomposition due to the fungicide application, there were no substantial effects by the treatments compared with the control. Again, decomposition was generally higher with nitroline than with CAN.

**Table 8.** Decomposition of residues, Colony Forming Units and macroconidia density as influenced by burial depth and treatment after 90 days of greenhouse test (greenhouse test II, III)

Burying depth	T*	Decomposition of residues (g) <sup>+</sup>		Colony Forming Units (CFU g <sup>-1</sup> )		Macroconidia density (× 10 <sup>6</sup> g <sup>-1</sup> )	
		Test II	Test III	Test II (sterilized)	Test III (not sterilized)	Test II	Test III
5 cm	T1	2.31	2.86	38	50	0.69	1.11
	T2	2.00	2.67	41	72	0.88	2.21
	T3	2.24	2.68	22	33	0.36	0.42
	T4	1.06	1.23	0	0	0.00	0.00
10 cm	T1	2.18	2.80	23	34	0.44	0.33
	T2	1.82	2.55	24	38	0.33	0.58
	T3	2.28	2.57	13	21	0.19	0.13
	T4	0.81	1.22	0	0	0.00	0.00
15 cm	T1	2.01	2.81	20	17	0.14	0.34
	T2	1.80	2.33	20	30	0.28	0.28
	T3	2.28	2.57	11	16	0.13	0.05
	T4	1.16	1.13	0	0	0.00	0.00
LSD <sub>0.05</sub>		n. s.	n. s.	4	7	0.27	0.38

\* T = Treatment (T1 = control; T2 = CAN application; T3 = nitroline application; T4 = Fungicide treatment).

<sup>+</sup> Difference between initial 5 g of residue and weight of sampling.

### 3.4 Colony Forming Units (CFU) and macroconidia density

In general, CFU levels and macroconidia density responded to the various treatments in a similar way. In greenhouse test I, the number of *F. graminearum* colonies formed on agar plates and the macroconidia density were consistently higher for wheat than for maize residues, but for the other greenhouse tests (II and III), more *F. graminearum* colonies and macroconidia developed on maize straw (Tables 5, 6). These differences were not observed following the fungicide treatment as neither *F. graminearum* colonies nor macroconidia developed (Tables 5-8). Compared to the control, the number of *F. graminearum* colonies and



macroconidia were significantly reduced after nitroline application. By contrast, CAN increased the number of colonies (Tables 5-8).

At the first sampling date no macroconidia were found at deeper incorporation levels (10 cm, 15 cm) independent of the treatment (Table 7). Both *F. graminearum* population indicators decreased with increasing incorporation depth (Tables 7, 8). Populations were lower after application of nitroline compared to CAN or the control treatment, but at comparatively low infestation levels, *e.g.* at the first sampling date and the deepest incorporation depth, these differences were not always significant. At the 5 cm depth, the number of colonies was usually increased above the control level as a result of application of CAN.

### 3.5 Correlations between measured traits

Correlation coefficients were not determined for the fungicide application treatment (T4), because no colonies or macroconidia developed (Table 9). The correlation between decomposition and CFU was always significant, but only during greenhouse test III we found significant correlations between decomposition and macroconidia density. Finally, there were strong correlations between CFU and macroconidia density when residue samples were analyzed before winter (greenhouse tests I, III), but not when evaluated in spring (greenhouse test II).

**Table 9.** Correlation coefficients for different traits characterising the populations of *F. graminearum* on crop residues dependent on treatment (T)<sup>1)</sup> (2 residue types, 3 burying depths, 3 sampling dates)

N = 18		Greenhouse test I		Greenhouse test II		Greenhouse test III	
		Decomposition	Colony Forming Units	Decomposition	Colony Forming Units	Decomposition	Colony Forming Units
Colony Forming Units	T1	0.67**	-	0.83***	-	0.57**	-
	T2	0.46*	-	0.77***	-	0.73***	-
	T3	0.55*	-	0.68**	-	0.69***	-
Macroconidia density	T1	0.45	0.92***	0.07	0.45	0.72**	0.79***
	T2	0.25	0.82***	- 0.04	0.45	0.51*	0.87***
	T3	0.43	0.92***	- 0.07	0.53*	0.67**	0.86***

<sup>1)</sup> T= Treatment (T1 = Control; T2 = CAN application; T3 = Nitroline application).

\* = significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$ ; \*\*\* significant at  $P < 0.001$

#### 4 Discussion

The use of the litter-bag technique, *i.e.* incubating organic substrates in fiberglass-mesh bags, for field experiments has been criticized because the access of soil microfauna and roots is restricted compared with the natural positioning of the substrate (ANDRÉN 1987). As our greenhouse tests did not involve plants and the bag mesh width was about 1 mm, soil microorganisms likely had sufficient contact with the residues in the bags (MALKOMES 1980; HEISLER 1994).

The slower decomposition of wheat residues compared to those of maize during greenhouse tests I and III can be explained by the higher C:N ratio of the wheat straw at harvest. The C:N ratio is closely correlated with the relative mineralization potential of organic substrates added to soils (WHITMORE and HANDAYANTO 1997). When C:N of wheat residues was on a level lower than maize, as with the residues sampled after eight months in spring 1999 for greenhouse test II, the difference in decomposability between the two residue types disappeared.

It was surprising that the application of additional N fertilizer, above all that of CAN, did not stimulate the decomposition process. Presumably, the level of mineral N in the soil used for greenhouse tests (15.7 ppm) was already sufficiently high for maximum residue decomposition. The slightly positive effect of nitrolime on decomposition likely can thus be attributed to components of this fertilizer other than the nitrogen.

We found a comparatively significant and recurring relationship between the decomposition of crop residues and CFU, an indicator of *F. graminearum* biomass. Irrespective of the treatment, *F. graminearum* developed in parallel with the mineralization of the residues. This is not surprising, as *F. graminearum* is known to utilize crop residues. If crop residues are the main nutrient (*i.e.* mainly carbon) source for fungus, it can be expected that as decomposition proceeds the correlation to the *F. graminearum* biomass might become negative due to a developing shortness of nutrient. However, this was not observed in our greenhouse tests in which the maximum decomposition of the buried residues was about 70%. Although the correlation between CFU and macroconidia density was usually significant, associations between residue decomposition and macroconidia density were only evident in greenhouse test III.

The suppression of *F. graminearum* development in residues, can be achieved by direct control with a fungicide, or by influencing decomposition by residue placement and nitrogen application. Our results indicated that soaking of the residues in a Folicur solution (a. i.= tebuconazole), not only substantially decreased the decomposition of crop residues, but also prevented fungal growth and development. Tebuconazole is known to be effective against *F. graminearum* (FEHRMANN and DIEHL 1989; JORDAN and HUTCHEON 1989). Based on our results one can conclude that it would be useful to suppress *F. graminearum* development by treating residues following harvest with relative high concentrations of an

effective fungicide. However, our results do not agree with MOLLENHAUER et al. (1999), who found that the application of fungicides on cereals has no effect on the rate of straw decomposition. This contradiction may be due to the large difference in fungicide concentration when this is applied by spraying on a standing crop versus soaking of the straw for 60 min in a fungicide solution.

The present study showed that the application of nitrolime reduced the population of *F. graminearum* on crop residues, but the N rate of application was comparatively high. This reduction can likely be attributed to metabolites of nitrolime formed in the soil, especially cyanamide, which have mycostatic effects. Additionally, urea is produced, which can reduce *F. graminearum* infestation compared with ammonium nitrate fertilizer (TEICH 1987). By contrast, fertilization with CAN promoted growth and development of *Fusarium* compared to the control, although the rate of decomposition did not increase. Possibly the population level of *F. graminearum* was stimulated by the additional nutrients added to in the soil.

Incorporation depth had a significant influence on crop residue decomposition and the population of *F. graminearum* in the residues. The decomposition of residues, the level of CFU and macroconidia density decreased with increasing incorporation depth of the residues. After 90 days of greenhouse tests (I - III), the burial of crop residues at 10 cm or at 15 cm reduced the number of colonies by 16-28% or 39-42%, respectively, compared with near-surface incorporation at 5 cm.

Conservation tillage systems, which maintain more crop residues near the soil surface, are becoming more widely used. The residues serve as ground cover to reduce soil erosion and act as a sink-source for plant nutrients (HUBBARD and JORDAN 1996). But residues are also involved in the propagation of *Fusarium* spp. Agronomic practices such as tillage, residue management, and crop rotation all play important roles in determining the risk of this disease, by influencing the type, amount and location of inoculum. COOK (1981) concluded that maximum burial of host crop residues is probably useful in *Fusarium* spp. control since inoculum is released only from the infested residue lying on the soil surface.

The population level of *F. graminearum* on residues can be assessed by counting colonies developing on agar plates or by counting macroconidia washed off from the residue surface. As there was not always a correlation between these two indicators of *F. graminearum* level, it may be necessary and useful to apply both methods simultaneously to assess *F. graminearum* populations on crop residues.

In summary, *F. graminearum* developed in parallel with the decomposition of crop residues in the soil. A decline in its population level due to a developing shortness of nutrient was not observed as long as more than 30% of the residue were still available. It is questionable whether Folicur applied at a conventional spray on the stubble and straw in the field would provide sufficient suppression on *F. graminearum*. Nitrolime applications may help to reduce *F. graminearum* on infested residues without detrimental effects on the decomposition process. Burying

*F. graminearum* infested crop residues deeper can effectively reduce inoculum density for a succeeding crop, yet as decomposition is slowed down, this may allow *F. graminearum* to survive for longer periods in the soil. In combination with soil preparation measures under applied condition these processes may support infectious of susceptible crop at later positions of the crop rotation.

## Acknowledgements

The authors gratefully acknowledge the technical support of the greenhouse tests by Dipl.-Biol. D. Hoehn and Mrs. Andrea Richter.

## Literature

- ANDRÉN, O.: Decomposition of shoot and root litter of barley, lucerne and meadow fescue under field conditions. -Swed. J. agric. Res. **17**, 113-122, 1987.
- AUFHAMMER, W., E. KÜBLER, H.-P. KAUL, W. HERMANN, D. HÖHN, C. YI: Infection with head blight (*F. graminearum*, *F. culmorum*) and deoxynivalenol concentration in winter wheat as influenced by N fertilization. -Pflanzenbauwiss. **4** (2), 72-78, 2000.
- BROWN, P. L., D. T. DICKEY: Losses of wheat straw residue under simulated conditions. -Soil Sci. Soc. Am. Proc. **34**, 118-121, 1970.
- COOK, R. J.: *Fusarium* disease of wheat and other small grains in North America. - In: NELSON P. E., TOUSSOUN T. A. and COOK R. J. (eds) *Fusarium: Diseases, Biology, and Taxonomy*, University Park, the Pennsylvania State University Press. pp. 39-52, 1981.
- DILL-MACKY, R.: *Fusarium* head blight: recent epidemics and research efforts in the upper midwest of the united states. -In: DUBIN H. J. et al. (eds) *Fusarium Head Scab: Global status and future prospects*. pp. 1-6, 1996.
- DILL-MACKY, R., R. K. JONES: The effect of previous crops residues and tillage on *Fusarium* head blight of wheat. -Plant Disease. **84**, 71-76, 2000.
- DORAN, J. W.: Tillage effects on microbiological release of soil organic nitrogen. -Texas Agric. Exp. Stn. Misc. Publ. College Station, Tx. 1987.
- DOUGLAS, C. L., R. R. ALLMARAS, P. E. RASMUSSEN, R. E. RAMIG, N. C. ROAGER: Wheat straw composition and placement effects on decomposition in dryland agriculture of the Pacific Northwest. -Soil Sci. Soc. Am. J. **44**, 833-837, 1980.
- FEHRMANN, H., T. DIEHL: Partielle Taubähigkeit chemisch behandeln. -Pflanzenschutz-Praxis **2**, 29-31, 1989.
- FERNANDEZ, M. R., J. M. C. FERNANDES: Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern and central Brazil. -Can. J. Plant Pathol. **12**, 289-294, 1990.

- GRACIA-GARZA, J. A., D. R. FRAVEL: Effect of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. -Phytopathology. **88**, 544-549, 1998.
- HAWARE, M. P., M. P. NENE, M. NATARAJAN: The survival of *Fusarium oxysporum* f. sp. Ciceri in the soil in the absence of chickpea. -Phytopath. Medit. **35**, 9-12, 1996.
- HEISLER, V. C.: Significance of microflora, mesofauna and macrofauna for the decomposition of straw in differently compacted arable soils. -Zool. Anz. **233**, 153-172, 1994.
- HERMANN, W., W. AUFHAMMER, E. KÜBLER, H.-P. KAUL: Auswirkungen von Ährenbefall mit *Fusarium graminearum* auf die Kornqualität von Winterweizen, Wintertriticale und Winterroggen. -Pflanzenbauwiss. **3**, 82-87, 1999.
- HOCKING, A. D., J. I. PITT: Dicloran-glycerol medium for enumeration of xerophilic fungi from low- moisture foods. -Plenum Press, New York and London, pp. 315, 1980.
- HOLLAND, E. A., D. C. COLEMAN: Litter placement effects on microbial and organic matter dynamics in an agroecosystem. -Ecology. **68**, 425-433, 1987.
- HUBBARD, V. C., D. JORDAN: Nitrogen recovery by corn from Nitrogen-15 labeled wheat residues and intact roots and soil. -Soil Sci. Soc. Am. J. **60**, 1405-1410, 1996.
- HUBER, D. M., R. D. WATSON: Crop residues, nitrogen, and plant disease. -Soil Sci. **100**, 302-308, 1965.
- JORDAN, V. W. L., J. A. HUTCHEON: Evaluation of an experimental fungicide against *Fusarium* disease of winter wheat in glasshouse and large pot experiments outside. -Report Long Ashton Research Station, 1989.
- MARTIN, R. A., J. A. MACLEOD, C. CALDWELL: Influences of production inputs on incidence of infection by *Fusarium* species on cereal seed. -Plant Disease. **75**, 784-788, 1991.
- MALKOMES, H.-P.: Strohrotteversuche zur Erfassung von Herbizid-Nebenwirkungen auf den Strohumsatz im Boden. -Pedobiologia. **20**, 417-427, 1980.
- MCMULLEN, M. P., R. W. STACK: Effects of isolation techniques and media on the differential isolation of *Fusarium* species. -Phytopathology. **73**, 458-462, 1983.
- MILLER, J. D.: Epidemiology of *Fusarium* ear diseases of cereals. In: MILLER J. D. and TRENHOLM H. L. (eds) Mycotoxins in grain. -Eagan Press, St. Paul, MN. pp. 19-36, 1994.
- MILLER, J. D., J. CULLEY, K. FRASER, S. HUBBARD, F. MELOCHE: Effect of tillage practice on *Fusarium* head blight of wheat. -Can. J. of Plant Pathol. **20**, 95-103, 1998.
- MOLLENHAUER, S., H. PESCHKE, A. FLAATTER: Abbauverhalten von fungizidhandbehandeltem Weizenstroh. -Agribiol. Res. **52**, 3-4, 1999.

- NYVALL, R. F.: Chlamydospores of *Fusarium roseum* 'Graminearum' as survival structures. -Phytopathology. **60**, 1175-1177, 1970.
- OBST, A., J. LEPSCHY-VON GLEISSENTHAL, G. HUBER: Zur gezielten Bekämpfung der Ährenfusarien bei Weizen–Beobachtungen und Versuchs-ergebnisse aus Bayern. -Gesunde Pflanzen. **44**, 40-47, 1992.
- OBST, A.: Untersuchungen zur Epidemiologie und Bekämpfung des Ährenparasiten *Fusarium graminearum* an Weizen. -Mitt. Biol. Bundesanst. Land- und Forstwirtschaft. **301**, 73, 1994.
- REIS, E. M.: Integrated disease management – The changing concepts of controlling head blight and spot blotch. In: Saunders DA (ed) Wheat for the nontraditional warm areas. -Proc. Int. Conf. CIMMYT, Mexico, D. F. pp. 165-177, 1990.
- SAS, 1989. SAS/STAT guide for personal computers, version 6 edition. SAS Inst. Inc., Cary.
- SALAS, B.: Effect of tillage and cropping systems on root rot of wheat. -Ph.D. Thesis. NDSU, pp. 230, 1991.
- SMILEY, R. W., R. J. COOK, R. I. PAPPENDICK: *Fusarium* foot rot of wheat and peas as influenced by soil applications of anhydrous ammonia and ammonia potassium azide solutions. -Phytopathology. **62**, 86-91, 1972.
- STURZ, A. V., C. C. BERNIER: Survival of cereal root pathogens in the stubble and soil of cereal versus noncereal crops. -Can. J. Plant Pathol. **9**, 205-213, 1987.
- STURZ, A. V., H. W. JOHNSTON: Characterization of *Fusarium* colonization of spring barley and wheat produced on stubble or fallow soil. -Can. J. Plant Pathol. **7**, 270-276, 1985.
- SU, H., A. H. C. VAN BRUGGEN, K. V. SUBBARAO: Spore release of *Bremia lactucae* on lettuce is affected by timing of light initiation and decrease in relative humidity. -Phytopathology. **90** (1), 67-71, 2000.
- SUMMERELL, B. A., L. W. BURGESS, T. A. KLEIN, A. B. PATTISON: Stubble management and the site of penetration of wheat by *Fusarium graminearum* group 1. -Phytopathology. **80**, 877-879, 1990.
- SUTTON, J. C.: Epidemiology of wheat head blight and maize ear rot caused by *F. graminearum*. -Can. J. Plant Pathol. **4**, 195-209, 1982.
- TEICH, A. H.: Less wheat scab with urea than with ammonium nitrate fertilizers. -Cereal Res. Commun. **15**, 35-38, 1987.
- TEICH, A. H.: Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp. -In: Chelkowski J (eds) *Fusarium* Mycotoxins, Taxonomy and Pathogenicity. Elsevier, Amsterdam. pp. 269-282, 1989.
- WANG, Y. Z.: Epidemiology and management of wheat scab in China. -In: DUBIN H. J. et al. *Fusarium* Head Scab: Global status and future prospects. pp. 97-105, 1996.
- WHITMORE, A. P., E. HANDAYANTO: Simulating the mineralization of N from crop residues in relation to residue quality. -In: CADISCH G. and GILLER K. E. (eds)

- Driven by nature. Plant litter quality and decomposition. CAB International, Wallingford (UK). pp. 337-348, 1997.
- WIESE, M. V.: Scab (head blight). -In: Compendium of Wheat Diseases. 2<sup>nd</sup> ed. American Phytopathological Society, St. Paul, MN. pp. 16-18, 1987.
- YI, C., H.-P. KAUL, E. KÜBLER, W. AUFHAMMER: Methodological problems in field investigations on *Fusarium graminearum* infection of wheat. -J. Plant Diseases and Protection. **107** (6), 583-593, 2000.

## Head blight (*Fusarium graminearum*) and deoxynivalenol concentration in winter wheat as affected by pre-crop, soil tillage and nitrogen fertilization

### Ähren-*Fusarium* (*Fusarium graminearum*) und Deoxynivalenol-Gehalt von Winterweizen in Abhängigkeit von Vorfrucht, Bodenbearbeitung und N-Düngung

Cuilin Yi\*, H.-P. KAUL, E. KÜBLER, K. SCHWADORF\*\*, W. AUFHAMMER

University of Hohenheim, Institute for Crop Production and Grassland Research, Fruwirthstr. 23, D-70599 Stuttgart, Germany, E-mail: yicuilin@uni-hohenheim.de

\* corresponding author

\*\* University of Hohenheim, The State Institute for Agricultural Chemistry, Emil-Wolff-Str. 14, D-70599 Stuttgart, Germany

Received 25 January 2001; accepted 12 April 2001

## Summary

*Fusarium* head blight (FHB), caused by *F. graminearum*, is increasing world-wide. *Fusarium* mycotoxins are a serious threat of health and a reliable control by fungicides is not possible, yet. The present study was conducted in order to evaluate the influence of different pre-crops and crop husbandry on FHB incidence in winter wheat test crops. In a 2-years factorial field experiment on the experimental station Ihinger Hof of the University of Hohenheim (480 m a. s. l., loam soil, 7,9 °C, 690 mm), inoculated pre-crops of maize or spring wheat were harvested for silage with only the stubble remaining in the field or for grain by combine with the whole straw remaining. Subsequently, crop residues were left on the soil surface or ploughed under before sowing winter wheat. Nitrogen fertilizer was applied to these test crops with calcium ammonium nitrate (CAN) or nitrolime. FHB was assessed by plot scores, by observations of disease incidence, disease severity and grain infection, indirectly via grain germination and by chemical deoxynivalenol (DON) analyses. The infection by FHB and the grain contamination with DON were similar after maize and spring wheat, either for silage or for grain, but the method of pre-crop inoculation by infected oat grains might have masked differences between pre-crops. The reductions of FHB incidence due to ploughing or nitrolime application were 27–32 % or 31–59 % compared with residues remaining on the surface or CAN fertilization, respectively. Contemporary reductions in DON were less consistent. The assessment of percent infected ears can be recommended as a comparatively fast method for FHB evaluation that showed significant correlations with DON concentration and grain germination, too. But a reliable estimation of DON concentrations is not possible on the basis of infection assessments. In conclusion, crop health can be supported by crop husbandry to some degree, but FHB cannot be reliably controlled in susceptible rotations with abundant sources of inoculum.

**Key words:** *Fusarium graminearum*; deoxynivalenol (DON); crop rotation; soil tillage; nitrogen fertilization

## Zusammenfassung

Ähren-*Fusarium*, verursacht durch *F. graminearum*, nimmt weltweit zu, *Fusarium*-Mycotoxine sind eine ernsthafte Gefahr für die Gesundheit, und eine zuverlässige Bekämpfung des Erregers mit Fungiziden ist



derzeit nicht möglich. Der vorliegende Beitrag untersucht den Einfluss von unterschiedlichen Vorfrüchten und von pflanzenbaulichen Anbaumaßnahmen auf das Auftreten von Ähren-*Fusarium* an Winterweizenbeständen. In einem zweijährig auf der Versuchsstation Ihinger Hof der Universität Hohenheim (480 m ü. NN, Lehm, 7,9 °C, 690 mm) durchgeführten faktoriellen Feldversuch wurden Mais oder Sommerweizen als Vorfrüchte künstlich inokuliert. Zur Differenzierung der Ernterückstände, die ein Infektionspotential für eine anfällige Nachfrucht darstellen, wurden die Bestände als Ganzpflanzen-Silage oder zur Körnernutzung geerntet. Die Rückstände verblieben auf der Bodenoberfläche oder sie wurden vor der Einsaat des als Testfrucht folgenden Winterweizens eingepflügt. Der Winterweizen wurde mit Kalkammonsalpeter (KAS) oder Kalkstickstoff (KS) gedüngt. Der Befall mit Ähren-*Fusarium* wurde anhand von Parzellenbonituren, von Erhebungen der Befallshäufigkeit, der Befallsstärke und des Kornbefalls beobachtet. Darüber hinaus wurde die Keimfähigkeit und der Deoxynivalenol (DON)-Gehalt des geernteten Kornguts erfasst. Der *Fusarium*-Befall und die Belastung des Winterweizens mit DON waren nach Mais und nach Sommerweizen und bei beiden Nutzungsformen ähnlich. Die Methode zur Inokulation der Vorfrüchte mit infizierten Haferkörnern könnte jedoch Vorfruchtunterschiede verdeckt haben. Das Reduktionsausmaß der Befallshäufigkeit aufgrund der wendenden Bodenbearbeitung bzw. des KS-Einsatzes betrug 27–32 % bzw. 31–59 %, jeweils im Vergleich zu nicht-wendender Bearbeitung bzw. zu KAS-Düngung. Gleichzeitige Rückgänge im DON-Gehalt waren weniger deutlich. Der Anteil befallener Ähren kann als eine verhältnismäßig schnelle Methode zur Befallserhebung empfohlen werden, die zudem nachweisbar mit dem DON-Gehalt und der Keimfähigkeit korrelierte. Die Korrelationskoeffizienten waren allerdings niedrig, so dass eine Abschätzung der DON-Konzentration anhand des Befalls nicht möglich ist. Die Untersuchungen zeigen, dass Ähren-*Fusarium* durch pflanzenbauliche Maßnahmen teilweise reduziert werden kann. In risikoreichen Fruchtfolgen mit hohem Infektionsdruck ist der Befall aber nicht zuverlässig zu begrenzen.

**Stichwörter:** *Fusarium graminearum*; Deoxynivalenol (DON); Fruchtfolge; Bodenbearbeitung; N-Düngung

## 1 Introduction

*Fusarium* head blight (FHB), caused by *Fusarium* spp., is at present one of the most important fungal diseases world-wide. In China, the USA, Canada, some European countries and also in south Germany, *F. graminearum* is the dominating *Fusarium* species which can cause severe seedling blight, crown rot, root rot and head blight in cereals, especially in wheat. Infected crops are often contaminated with fungal metabolites which are toxic for human beings and animals (SUTTON 1982; JENKINSON and PARRY 1994; WANG 1997; MIEDANER 1997; DILL-MACKY and JONES 2000). *F. graminearum* produces a variety of mycotoxins, deoxynivalenol (DON) and its derivatives were most often found in wheat (TANAKA et al. 1990; SCOTT et al. 1996). Among 114 isolates of *F. graminearum* collected from soils or cereals on a world-wide basis, 95 % were able to produce DON (MIROCHA et al. 1989). In different countries, legal thresholds exist for *Fusarium* toxins in food and feed, e. g., in Canada DON concentration < 2 or < 5 mg kg<sup>-1</sup>, in the USA DON < 1 or < 5–10 mg kg<sup>-1</sup> in food or feed, respectively, and in Austria DON < 0.5 mg kg<sup>-1</sup> in food (GILGENBERG-HARTUNG 1999). Besides the contamination with toxic metabolites, the germination percentage of infected grains is substantially reduced. With about 92 % of infected grains, germination was below 20 %, and the germination of shrivelled grains due to *Fusarium* infection was none (BECHTEL et al. 1985).

MESTERHÁZY (1997a, b) found close correlations between visual head symptoms of FHB in wheat and the percentage of infected grains ( $r = 0.87\text{--}0.99$ ). The correlations of visual symptoms with toxin (DON) concentrations or yield losses were similar ( $r = 0.89\text{--}0.96$  or  $r = 0.80\text{--}0.97$ , respectively). HUI et al. (1997) confirm comparatively close correlations between disease incidence and DON concentration, but the relationship between disease incidence and disease severity was less pronounced. Disease incidence on individual ears and disease severity, assessed by the number of infected spikelets per ear, reflect different, only partly overlapping processes during crop infection (WILCOXSON et al. 1992). HERMANN et al. (1998) reported substantially lower correlations between visual scorings on a whole plot basis (scores of 1–9) and the percentage infected grains ( $r = 0.45$ ) or DON concentration ( $r = 0.28$ ).

The control of FHB by fungicides is an unsolved problem, yet. MILUS and PARSONS (1994) assume that some fungicides are effective only at low infection levels. Fungicides containing tebuconazole show efficacy against FHB and yields can be increased by 35 % due to application of the fungicide Folicur (a. i.: tebuconazole) (FEHRMANN and DIEHL 1989; JORDAN and HUTCHEON 1989; MESTERHÁZY 1997b). But the effect depends much on weather conditions (OBST et al. 1992). It may even happen that the application of fungicides which are not intended for FHB control is associated with increased DON accumulation in grain (HART and WARD 1997; HERMANN et al. 1999; OBST and GAMMEL 2000).

Cultural practices and crop husbandry can influence the ear infection with *Fusarium* and the subsequent accumulation of mycotoxins (SNIJDERS 1994; MILLER et al. 1998). The increased incidence of FHB in many regions world-wide has been attributed to high percentages of wheat and maize in crop rotations, reduced tillage and prolonged residue retention on the soil surface (STURZ and JOHNSTON 1985; SUMMERELL et al. 1990; DILL-MACKY 1997; WANG 1997; MILLER et al. 1998; DILL-MACKY and JONES 2000). In maize, *F. graminearum* causes foot rot symptoms and maize stubble is an important source of FHB inoculum for wheat (LBP 1997; BECK and LEPSCHY 2000). TEICH and NELSON (1984) found that the average incidence of FHB on wheat following maize was up to seven times higher than that on wheat following wheat, barley, oats or soybean. According to SEAMAN (1982), maize and wheat grown in rotation leave abundant residues which are a primary source of inoculum. Burying these residues may reduce the primary inoculum. Consequently, DILL-MACKY & JONES (2000) found that FHB severity was lower in moldboard ploughed plots than in either chisel ploughed or no-till plots.

High soil mineral nitrogen concentrations can promote FHB incidence in wheat (TEICH 1989; MARTIN et al. 1991; AUFHAMMER et al. 2000). The type of nitrogen compound is of importance, too. The incidence and severity of *Fusarium* root-rot diseases of winter wheat was higher after fertilization with ammonium ( $\text{NH}_4$ ) compared with nitrate ( $\text{NO}_3$ ), and wheat fertilized with urea ( $\text{CO}(\text{NH}_2)_2$ ) showed less FHB symptoms than wheat fertilized with ammonium nitrate (HUBER and WATSON 1965; SMILEY et al. 1972; TEICH 1987; MARTIN et al. 1991). Liming reduced the severity of *Fusarium* wilt (*F. oxysporum*) and nitrate-nitrogen applied in addition to calcium hydroxide decreased wilt even more (WOLTZ and ENGELHARD 1972). Nitrolime was shown to reduce growth and development of *F. graminearum* on the crop residues of maize and wheat in greenhouse pot incubation experiments (YI et al. 2000b).

Under these circumstances, crop rotation and crop husbandry are the only clues to support the health of susceptible crops, particularly of wheat, and thus the health of food and feed. Therefore, the present study was conducted to answer the following questions:

- Does the infection level of winter wheat crops with *F. graminearum* differ after infected pre-crops of either maize or wheat?
- Can we control FHB infection by reducing the crop residues of the pre-crops on the soil surface due to silage use or by ploughing under the residues of combined crops?
- Does the fungi-static effect of nitrolime, as observed with *F. graminearum* in pot experiments (YI et al. 2000b), reduce FHB infection in the field compared with CAN fertilization?
- Which of several methods can be recommended for a fast, cheap and reliable assessment of the infection level and the toxin contamination of a wheat crop? The infection of wheat with *F. graminearum* can be assessed by different traits: By easily obtainable plot scores, by comparatively time consuming investigations of disease incidence, disease severity and grain infection, indirectly via grain germination and, finally, on the basis of very expensive, but also most relevant analyses of DON concentration.

## 2 Materials and methods

### 2.1 Experimental design

A factorial field experiment was conducted twice at the experimental station Ihinger Hof (480 m a. s. l., 7.9 °C, 690 mm, loamy soil) of the University of Hohenheim in 1997–99 in a split-plot design with four replicates (Table 1, 2). Different pre-crops were arranged on main plots with treatments on sub-plots of 8 m × 8 m size.

Table 1. Crop rotations in field experiments  
Tab. 1. Fruchtfolgen in den Feldversuchen

Experimental period	1997–1998		1998–1999	
1997	Maize*	Spring wheat*	Pre-experimental period	
1998	Winter wheat (Test crop)	Winter wheat (Test crop)	Maize*	Spring wheat*
1999	Post-experimental period		Winter wheat (Test crop)	Winter wheat (Test crop)

\* Maize and spring wheat were planted as pre-crops and inoculated with *F. graminearum* infected oat grains

Table 2. Factorial design of experiment  
Tab. 2. Versuchsfaktoren und Faktorstufen

Experimental periods	1997/98 and 1998/99
Pre-crops	P1: Maize, cv. ‘Helix’, sown April 1997; May 1998 P2: Spring wheat, cv. ‘Hanno’, sown March 1997; March 1998
Treatments of test crops	T1: Pre-crops for silage, only stubble left on the soil surface; Non-inversion tillage (chisel plough); CAN*, 40 kg N ha <sup>-1</sup> at beginning of growing season + 60 kg N ha <sup>-1</sup> before stem elongation + 60 kg N ha <sup>-1</sup> at heading T2: Pre-crops for grain, straw residues ploughed into the soil; Inversion tillage (moldboard plough ≈ 20 cm depth); CAN as with T1 T3: Pre-crops for grain, straw residues left on the soil surface; Tillage as with T1; CAN as with T1 T4: Pre-crops for grain, straw residues left on the soil surface; Tillage as with T1; Nitrolime, 100 kg N ha <sup>-1</sup> at beginning of growing season + CAN, 60 kg N ha <sup>-1</sup> at heading

\* CAN = calcium ammonium nitrate

Maize (cv. ‘Helix’) or spring (cv. ‘Hanno’) wheat were planted as pre-crops in rotations with winter wheat in spring 1997 and 1998. The pre-crops were inoculated by *F. graminearum* infected oat grains in order to simulate the natural infection process. For this purpose, 10 g m<sup>-2</sup> of infected grains were broadcast in the maize crops on the soil at 5-leaves stage and additional 5 g m<sup>-2</sup> at the beginning of tassel emergence. Correspondingly, infected oat grains were broadcast in the spring wheat crops at the end of tillering (10 g m<sup>-2</sup>), at the beginning of stem elongation (10 g m<sup>-2</sup>) and at heading stage (5 g m<sup>-2</sup>) (Obst et al. 1992; Yi et al. 2000a). After harvest of maize and spring wheat, test crops of winter wheat, cv. ‘Flair’, were sown on 28 October 1997 and 19 November 1998, respectively.

In parallel with the main wind direction, i. e., from west to east, test-plots were spaced 25 m apart with winter wheat crops in between in order to avoid inter-plot disease spread. Rectangular to the main wind direction, i. e., from north to south, 8 m wide isolation strips of tall growing winter rape were planted (Yi et al. 2000a). Winter rape had been sown on 5 September 1997 and 25 August 1998, respectively.

There were four treatments (T1–T4) in the field experiment (Table 2). Pre-crops were either harvested for silage with only the stubble remaining in the field (T1) or they were harvested for grain by

combine scattering the straw completely on the soil (T2-T4). Subsequently, the residues were either left on the soil surface (T1, T3, T4) or ploughed under (T2) before sowing the winter wheat test crops. Nitrogen fertilizer was applied to these test crops at a rate of 160 kg N ha<sup>-1</sup> either with calcium ammonium nitrate (CAN, T1-T3) or 100 kg N ha<sup>-1</sup> of CAN were substituted by nitrolime (T4). In order to prevent a severe incidence of fungal diseases other than FHB, a fungicide not effective against *Fusarium* spp. (1.25 L ha<sup>-1</sup> Sportak Delta, a. i.: cyproconazole, prochloraz) was applied against root rot, mildew and leaf spot during stem elongation.

2.2 Observations of inoculated pre-crops in the field

The stem infection of the pre-crop maize was assessed at dough stage (BBCH85) from one row of 40 plants cut at the stem basis on each plot. From these samples, the number of infected stems was counted (KRÜGER 1985). Similarly, the ear infection of the pre-crop spring wheat was assessed at soft dough stage (BBCH85) from random samples of 50 ears per plot. From these samples, the number of visibly infected ears (disease incidence) was counted.

2.3 Observations of test crops in field and laboratory

In the field, the ear infection of test crops was studied four times at medium (BBCH75) and late milk ripeness (BBCH77) as well as at early (BBCH83) and medium dough ripeness (BBCH85) from random samples of 50 ears per plot (Table 3). From these samples, both the number of infected ears (disease incidence) and the number of infected spikelets were counted, which allowed for the calculation of disease severity, i. e., the number of infected spikelets per infected ear. Unlike other authors (WILCOXON et al. 1992), who relate the number of infected spikelets to the number of ears under observation, we chose the number of infected ears as the denominator in order to separate more distinctly the infection of individual ears and the disease spread within these ears. Additionally, the infection was scored (1–9) on a whole plot basis (MIEDANER 1986), but at growth stages later than BBCH83 scoring was impossible because the proceeding senescence of the test crops masked the FHB symptoms. At full ripeness (BBCH97), the crops were finally harvested by combine.

Under laboratory conditions, the grain infection was estimated. Briefly, 100 grains from final harvest without surface sterilization were soaked in water, incubated for 3 days and the number of red colored grains was counted (BAIER et al. 1998). Furthermore, the grain from final harvest was used to

Table 3. Measured and calculated traits  
Tab. 3. Erfasste und errechnete Parameter

Plot score	Ear infection was scored (1–9) on a whole plot basis (MIEDANER 1986) at BBCH75, BBCH77 and BBCH83.
Ear and spikelet infection	50 ears were sampled at random from each plot at BBCH75, BBCH77, BBCH83 and BBCH85. From these samples, infected ears and spikelets were counted. Disease incidence = Infected ears (% , based on 50 ears) Disease severity = Infected spikelets per infected ear (number, based on 50 ears)
Grain infection	100 grains from final harvest were soaked in water and incubated for 3 days (BAIER et al. 1998). Grain infection = Red colored grains (% , based on 100 grains)
Grain germination	Germination percentage of 100 grains from final harvest was determined (AOSA 1978). Grain germination = Germinated grains (% , based on 100 grains)
Deoxynivalenol (DON) concentration	Subsamples (20 g) of ground grain material were analyzed for DON concentration (µg kg <sup>-1</sup> original substance) at BBCH75, BBCH83 and at final harvest by gas chromatography and mass spectrometry (SCHWADORF & MÜLLER 1991).

determine the germination percentage as an indirect indicator for the infection level of individual grains (AOSA 1978).

For analysis of deoxynivalenol (DON) concentrations, samples of winter wheat grain from BBCH75, BBCH83 and from final harvest were ground (1 mm sieve width). The ground material was thoroughly mixed and further subsampled for analysis. Briefly, a subsample (20 g) was extracted, cleaned up, derivatized and measured by gas chromatography and mass spectrometry according to a method described by SCHWADORF and MÜLLER (1991).

## 2.4 Statistical analysis

Plot scores, disease incidence and severity, grain infection, germination and DON concentration results were analyzed according to the factorial design of the field experiment separately for both experimental periods and all sampling dates using the General Linear Model (GLM) procedure of SAS (SAS 1989). In case of significant effects ( $P < 0.05$ ), Least Significant Differences ( $LSD_{0.05}$ ) were calculated for the separation of means. Due to comparatively large residual variances, differences in disease incidence and severity were not statistically significant when the data from each sampling date were analyzed individually. In order to obtain more stable mean values, both traits were averaged across four sampling dates.

The relationships between the different traits measured to characterize the infection of wheat with *F. graminearum* were assessed by correlation analysis. Pearson correlation coefficients were calculated by the procedure CORR from SAS. This analysis was performed separately for both experimental periods.

## 3 Results

### 3.1 Environmental conditions for test crop infection with *F. graminearum*

Climate conditions during the periods of investigation in 1998 and 1999 are given in Figure 1. The data suggest that the precipitation conditions were more favourable for infection of *F. graminearum* in 1999, while in 1998 the infection period, i. e., flowering time, was comparatively dry. In total, the sum of precipitation from June to August was much higher in 1999 while the temperature was similar in both years.

In both years, the inoculated pre-crop maize did not show any infection symptoms at the stem base. On the contrary, the pre-crop spring wheat was obviously infected with FHB. The disease incidence on spring wheat at BBCH85 was on average 11 % in 1997 and 53 % in 1998.

### 3.2 Results of field observations

The analyses of variance of the different traits indicating FHB infection of the winter wheat test crops did not reveal regularly significant main effects of pre-crops nor interactions of pre-crops with treatments, indicating that the infection was similar after maize and spring wheat. Therefore, the presentation of results is restricted to treatment effects.

Infections of the winter wheat test crops occurred obviously in both years. The plot scorings of FHB infection of winter wheat show that in both years the infection was on a similar level (Table 4). This infection level, however, was only moderate. It did not exceed a score of 4, i. e., bleaching of ears was 25 % at most. When crop residues were left on the soil surface untreated, either after silage (T1) or after grain harvest (T3), the infection was higher than after ploughing (T2) or with nitrolime application (T4). The more detailed investigations of disease incidence and severity revealed the same effects (Table 5). The infection level can be ranked in the sequence  $T1 > T3 > T2, T4$ .

### 3.3 Results of laboratory observations

Unlike the field observations, the observation under laboratory conditions revealed occasionally interaction between pre-crops and treatments. In general, the grain infection results confirm the field

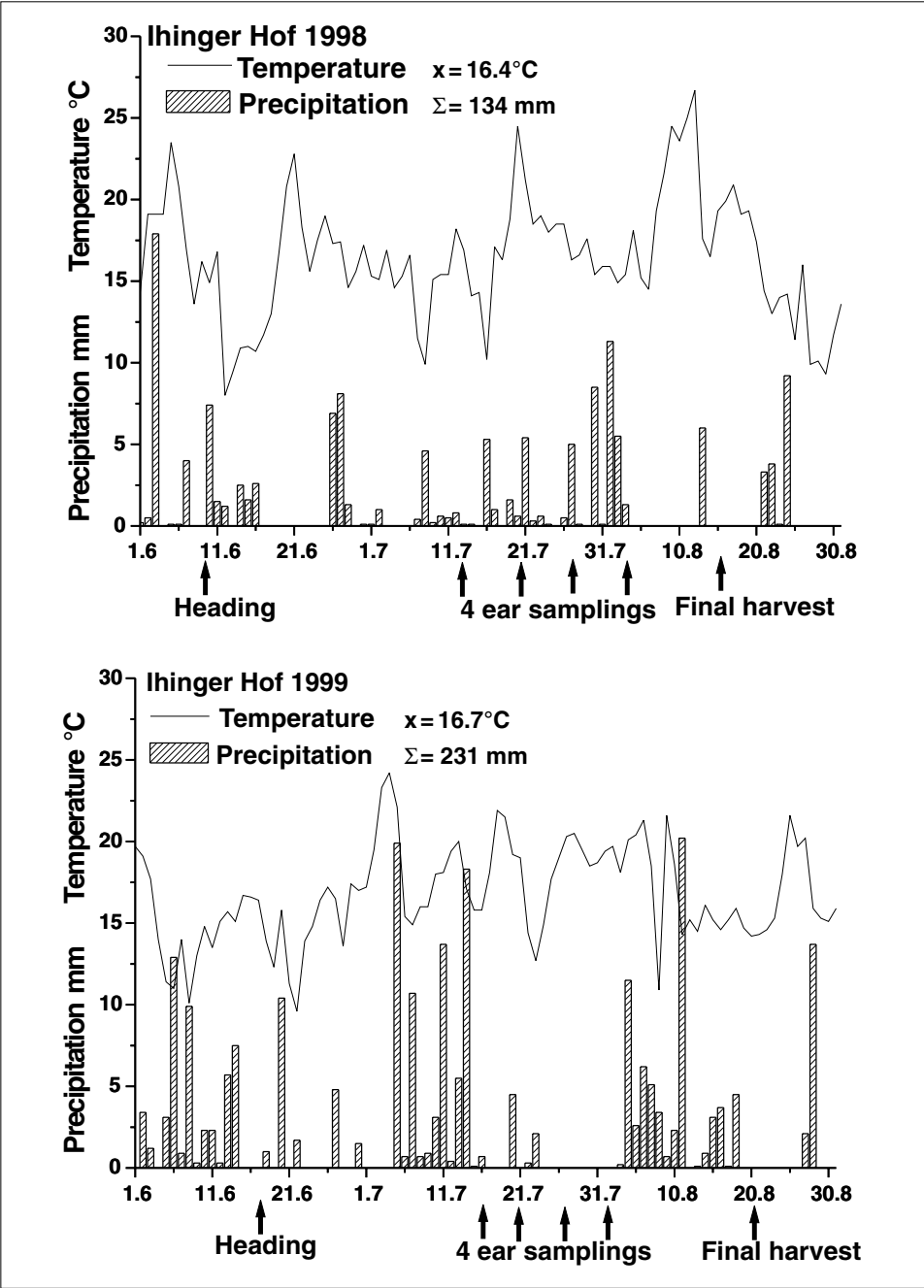


Fig. 1. Daily air temperature ( $^{\circ}\text{C}$ ) and precipitation (mm) from June to August as well as dates of heading, ear sampling and final harvest.  
Abb. 1. Tageswerte von Lufttemperatur ( $^{\circ}\text{C}$ ) und Niederschlag (mm) für Juni bis August sowie Zeitpunkte des Ährenschiebens, Probennahme- und Druschtermine.

Table 4. Plot scores (1–9) of FHB on winter wheat dependent on treatments at sequential sampling dates

Tab. 4. Befallsbonituren (1–9) von Ähren-*Fusarium* an Winterweizen in Abhängigkeit von den Behandlungen zu mehreren Untersuchungsterminen

Treatment	1998			1999		
	BBCH 75	BBCH 77	BBCH 83	BBCH 75	BBCH 77	BBCH 83
T1	2.4	3.2	3.3	3.6	2.7	2.8
T2	1.9	2.4	2.5	2.3	2.2	2.3
T3	1.9	2.7	3.1	3.2	2.5	3.2
T4	1.4	2.3	2.5	2.4	2.3	2.3
LSD <sub>0,05</sub>	0.5	n. s.	n. s.	0.5	0.3	0.4

Table 5. Disease incidence, disease severity (means from four sampling dates) and grain infection of *Fusarium graminearum* on winter wheat dependent on pre-crops (only grain infection in 1999) and treatments

Tab. 5. Befallshäufigkeit und Befallsstärke (Durchschnitt von vier Untersuchungsterminen) sowie Kornbefall mit *F. graminearum* an Winterweizen in Abhängigkeit von den Vorfrüchten (nur Kornbefall 1999) und den Behandlungen

Treatment	Disease incidence (%)		Disease severity		Grain infection (%)		
	1998	1999	1998	1999	1998	1999	
						P1	P2
T1	16.9	16.9	1.8	1.8	17.3	20.0	16.5
T2	5.9	10.9	1.1	1.3	8.3	10.8	12.5
T3	8.1	16.1	1.4	1.6	16.3	21.8	15.0
T4	3.4	11.1	1.2	1.4	5.8	9.8	12.5
LSD <sub>0,05</sub>	4.0	4.9	0.6	0.4	n. s.	3.8	

observations (Table 5). In 1999, however, the differences between treatments (T1, T3 > T2, T4) were more pronounced after the pre-crop maize (P1).

During early phases of winter wheat grain development (BBCH75), no consistent differences in DON concentrations were found (Table 6). In 1999, differences in DON concentrations between pre-crops or treatments were generally only small. In 1998, the treatments with the higher infection level (T1, T3) showed also comparatively high DON concentrations in grain. This year, the winter wheat test crops after maize for silage use were contaminated with DON most severely.

Germination percentages of harvested grain were determined as an indirect indicator for the infection level of individual grains (Table 7). Compared with tillage (T2) or nitrolime application (T4), germination percentages were reduced after silage (T1) or grain harvest (T3) of the pre-crops, respectively, without any subsequent treatment. The detrimental effect of the pre-crops harvested for grain (T3) was significant only in 1998.

3.4 Correlations between measured traits

The correlation coefficients between different traits characterizing the FHB infection of wheat were generally higher in 1998 compared with 1999 (Table 8). Only disease incidence showed consistent correlations with most of the other traits, including DON concentration and grain germination, in both years. Additionally, the DON concentration was significantly related to grain germination.

Table 6. DON concentration ( $\mu\text{g kg}^{-1}$ ) of winter wheat grain dependent on pre-crops and treatments at sequential sampling dates

Tab. 6. DON Konzentration ( $\mu\text{g kg}^{-1}$ ) im Korngut von Winterweizen in Abhängigkeit von den Vorfrüchten und den Behandlungen zu mehreren Untersuchungsterminen

Treatment		BBCH 75		BBCH83		Harvested grains	
		P1	P2	P1	P2	P1	P2
1998	T1	1736	1185	3950	1251	1376	308
	T2	1264	1392	1438	1632	348	248
	T3	1041	1339	1092	2103	569	293
	T4	727	857	1259	641	293	192
LSD <sub>0.05</sub>		n. s.		1919		404	
1999	T1	801	539	872	799	549	943
	T2	461	440	551	126	523	263
	T3	874	1119	549	911	614	686
	T4	553	485	752	978	516	695
LSD <sub>0.05</sub>		n. s.		n. s.		n. s.	

Table 7. Germination percentage (%) of harvested winter wheat grain dependent on treatments

Tab. 7. Keimfähigkeit (%) des Erntegutes von Winterweizen in Abhängigkeit von den Behandlungen

Treatment	1998	1999
T1	85.1	83.6
T2	90.1	88.8
T3	86.6	87.2
T4	93.6	91.0
LSD <sub>0.05</sub>	3.5	4.7

4 Discussion

During our field experiments, pre-crops of maize or spring wheat in rotations with winter wheat were inoculated by *F. graminearum*-infected oat grains. The intention was to simulate the natural inoculum source of crop residues and the infection process originating from these residues. But in both years, the pre-crop maize did not show any infection symptoms of *Fusarium* foot rot at the stem base. On the contrary, the ears of spring wheat were clearly infected with *F. graminearum*. The missing infection of maize may have been due to the smaller amount of inoculum that had been applied to maize (15 g m<sup>-2</sup>) compared with wheat (25 g m<sup>-2</sup>) or the different dates of inoculation. Presumably, the inoculation of maize before flowering was too early for stem infection. Additionally, the maize cv. ‘Helix’ is only to a low degree susceptible to *Fusarium* foot rot (BSA 1997). Beyond these arguments, the artificial inoculation of maize in fields without a soil-borne infection potential might be a methodical problem that deserves further investigation.

The infection level of the winter wheat test crops did not exceed 20 % disease incidence corresponding to a plot score of 3–4 according to MIEDANER (1986). During the investigation period from BBCH75–85, hardly any increase of disease incidence was observed (data not presented), hence the calculation of more stable means across the four sampling dates, i. e., across 200 ears instead of 50 ears per individual sampling, seemed to be justifiable. Also, the deoxynivalenol (DON) concentrations in the grain material of winter wheat were on a moderate level compared with the concentrations which were observed after spray inoculation of ears with spore suspensions at heading of crops (AUFHAMMER et al. 2000). However,



Table 8. Correlation coefficients for different traits measured to characterize FHB infection  
Tab. 8. Korrelationskoeffizienten für verschiedene Merkmale des Befalls mit Ähren-*Fusarium*

	Plot score (1–9)		Disease incidence (%)		Disease severity		Grain infection (%)		DON (µg kg <sup>-1</sup> )	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Disease incidence	0.50*** (n = 80)	0.17 (n = 96)	–	–	–	–	–	–	–	–
Disease severity	0.33*** (n = 80)	0.08 (n = 96)	0.52*** (n = 112)	0.44*** (n = 128)	–	–	–	–	–	–
Grain infection	0.44** (n = 32)	–0.06 (n = 32)	0.42* (n = 32)	0.29 (n = 32)	–0.07 (n = 32)	0.25 (n = 32)	–	–	–	–
DON	0.18 (n = 80)	0.24 (n = 96)	0.29* (n = 80)	0.43*** (n = 96)	0.24 (n = 80)	0.21 (n = 96)	0.41* (n = 32)	0.21 (n = 32)	–	–
Grain germination (%)	–0.71*** (n = 32)	–0.14 (n = 32)	–0.80*** (n = 32)	–0.54*** (n = 32)	–0.02 (n = 32)	–0.43 (n = 32)	–0.55*** (n = 32)	–0.28 (n = 32)	–0.76*** (n = 32)	–0.40* (n = 32)

a threshold value of 1 mg DON kg<sup>-1</sup>, which is expected for food grain in Europe, was occasionally exceeded. Obviously, DON concentrations at that level are easily obtained under natural infection conditions (MÜLLER and SCHWADORF 1993; JONES and MIROCHA 1999).

As in earlier studies (HERMANN et al. 1998; AUFHAMMER et al. 2000), the residual variability of disease incidence and DON production on slightly infected crops was comparatively large. The isolation widths between adjacent sub-plots of 25 m in parallel with the main wind direction and 8 m rectangular to the main wind direction should have reduced inter-plot disease spread to a high degree, although it cannot be completely eliminated (FERNANDO et al. 1997; YI et al. 2000a). With view to the large variability of infection even between different grains within one individual ear (WEINERT and WOLF 1999), statistical significance can be obtained only with sufficiently large samples.

The results of this study prove that the infection of winter wheat crops with *F. graminearum* and the subsequent consequences for the grain contamination with DON and for grain germination are affected by soil tillage and nitrogen fertilization, but the different pre-crops – maize or spring wheat, either for silage or for grain – showed hardly any effect. This is in contradiction with other investigations which suggest that wheat following maize in a rotation system is more prone to severe FHB infections than wheat after wheat (HOFFER et al. 1918; HOLBERT et al. 1919; KOEHLER et al. 1924; SNYDER and NASH 1968; SEAMAN 1982; SUTTON 1982; TEICH and NELSON 1984). Maybe comparatively low infection levels after maize were due to the missing infection of maize stems. On the other hand, although the maize was not visibly infected, there were no differences in infection level of the test crop winter wheat observed between the pre-crops maize or wheat and in 1 year the DON concentration in wheat grain was even higher after maize than after wheat for silage.

It cannot be decided, whether the primary sources of inoculum were really the crop residues or the oat grains, which were

still visibly lying on the soil surface at harvest of the pre-crops. The considerable infection level after maize suggests an eventual role of the inoculation material of oat grains. Moreover, the slightly higher infection level after silage use of the pre-crops compared with grain use may have resulted from the easier propagation of *Fusarium* spores without large amounts of straw on the soil. There were different possible sources of inoculum, including the oat grains, the wheat chaff or the maize stubble, that might have been covered on the soil surface.

Crop residues in no-till plots can provide a significant source of *Fusarium* inoculum (MILLER et al. 1998). It seems that comparatively small amounts of residues after silage harvest of maize or spring wheat were already sufficient for a high level of infection. But again, we cannot exclude that the oat grains applied for inoculation of the pre-crops were an important source of inoculation of the test crops. *F. graminearum* can persist on residues under both till and no-till conditions and infections can occur in subsequent years. However, ploughing under of residues before sowing a subsequent crop reduces the prevalence of FHB in the following year. A greenhouse pot incubation study showed that incorporation depth of crop residues has a significant influence on the growth of *F. graminearum* on the crop residues. The number of fungal units on the residues decreased with increasing incorporation depth. After 90 days of incubation, the burial of crop residues at 10 cm or at 15 cm reduced the number of *Fusarium* colonies by 16–28 % or 39–42 %, respectively, compared with surface incorporation at 5 cm (Yi et al. 2000b). Although under field conditions a deep burial might conserve pre-crop residues for a long period due to lower soil temperatures and less aeration deep in the soil, incorporating crop residues as completely and as early as possible may reduce disease incidence and severity for the succeeding crop to the highest degree (MARTIN and JOHNSTON 1982; MILLER et al. 1998; DILL-MACKY and JONES 2000). Our results underline the favourable effect of removing the crop residues of the pre-crops from the soil surface by ploughing them into the soil. Ploughing (T2) reduced FHB incidence compared with surface residues (T3) by 27–32 % in 1998 and 1999, respectively. At the same time, disease severity was reduced by 19–21 %.

The importance of the amount of nitrogen and the type of nitrogen fertilizer for the incidence of *Fusarium* spp. was stressed by many authors (HUBER and WATSON 1965; SMILEY et al. 1972; WOLTZ and ENGELHARD 1972; TEICH 1987, 1989; MARTIN et al. 1991; AUFHAMMER et al. 2000; Yi et al. 2000b). In our present study, nitrolime (T4) showed a fungi-static effect in the field that was similar to previous observations with *F. graminearum* in pot experiments (Yi et al. 2000b). It reduced FHB incidence compared with CAN fertilization (T3) by 31–59 % in 1999 and 1998, respectively. Disease severity was also reduced, but only by 10–13 %.

The infection of wheat with *F. graminearum* can be characterized by different direct or indirect indicators (SUTTON 1982; TANAKA et al. 1990; MIROCHA et al. 1989; SCOTT et al. 1996; JENKINSON and PARRY 1994; WANG 1997; MIEDANER 1997; DILL-MACKY and JONES 2000). From the results of the present study, we can recommend to assess disease incidence, i. e., the percentage of infected ears, because it is a comparatively fast method that showed close correlations with most of the other traits under investigation, particularly with those most relevant for grain utilization, i. e., DON concentration and grain germination. But the correlation coefficients were only moderately high and with DON they were always  $r < 0.5$ . Thus, a reliable estimation of DON contamination is impossible on the basis of infection assessments. Due to considerable error variances under natural infection conditions, the sample size should be above 50 ears per plot in order to obtain representative results.

Plot scores, which are most easily obtained, rendered a consistent disease assessment only in 1 year, although the scores were in general agreement with the other indicators of FHB. Also, the very time-consuming counting of infected spikelets per ear or the determination of grain infection in the laboratory did not allow for a better description of the infection level. Grain germination did reflect the differences in infection as well as in DON concentration, but the variability in germination due to the experimental treatments was comparatively small.

In conclusion, DON concentrations in winter wheat grain produced under natural infection conditions for *F. graminearum* can easily exceed values that are critical for human consumption. The correlation between FHB infection and the contamination of grain with DON is only weak which makes a chemical analysis of the toxin unavoidable. Under the conditions in our experiments, the species of the pre-crop – either maize or spring wheat – did not affect the infection level, but the method of inoculation might have masked differences between pre-crops. Both approaches to support

crop health by crop husbandry – by ploughing or by application of nitroline on the crop residues – were successful to some degree, but they are not suitable to guarantee a harmless infection and contamination level under all circumstances. Yet, FHB is not reliably controllable in susceptible rotations with abundant sources of inoculum.

## Acknowledgements

The authors gratefully acknowledge the technical support of the DON analyses by the State Institute for Agricultural Chemistry at the University of Hohenheim, namely by Prof. Dr. H. Schenkel, Dr. B. Eckstein and Mrs. A. Eschle.

## Literature

- AOA (Association of Official Seed Analysts): Rules for testing seeds. – Proc. Ass. official Seed Analysts **60**, 39, 1978.
- AUFHAMMER, W., E. KÜBLER, H.-P. KAUL, W. HERMANN, D. HÖHN, C. YI: Infection with head blight (*F. graminearum*, *F. culmorum*) and deoxynivalenol concentration in winter wheat as influenced by N fertilization. – Pflanzenbauwiss. **4**, 72–78, 2000.
- BAIER, W. H., G. BEESE, E. ROICK, E. ALBERT, C. GEBHART, H. ZIMMERMANN: Brauweizenbericht Sachsen – Sorteneignung, Anbauggebiete, Anbaubedingungen. – Schriftenr. Sächs. Land. anst. Landw. **3**, 65, 1998.
- BECHTEL, D. B., L. A. KALEIKAU, L. A. GAINES, L. M. SEITZ: The effects of *Fusarium graminearum* infection on wheat kernels. – Cereal Chem. **62**, 191–197, 1985.
- BECK, R., J. LEPSCHY: Ergebnisse aus dem Fusarium-Monitoring 1989–1999 – Einfluss der produktionstechnischen Faktoren Fruchtfolge und Bodenbearbeitung. – LBP (Bayer. Land. anst. Bodenkultur u. Pfl. bau) **3**, 39–47, 2000.
- BSA (Bundessortenamt): Beschreibende Sortenliste – Getreide, Mais, Ölfrüchte, Leguminosen (groszkörnig) und Hackfrüchte (ausser Kartoffeln). – Verlag Landbuch, Hannover, 1997.
- DILL-MACKY, R.: *Fusarium* head blight: recent epidemics and research efforts in the upper midwest of the United States. – In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: Global status and future prospects, pp. 1–6 CIMMYT, Mexico, 1997.
- DILL-MACKY, R., R. K. JONES: The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. – Pl. Dis. **84**, 71–76, 2000.
- FEHRMANN, H., T. DIEHL: Partielle Taubährigkeit chemisch behandeln. – Pfl. Schutz-Praxis **2**, 29–31, 1989.
- FERNANDO, W. G. D., T. C. PAULITZ, W. L. SEAMAN, P. DUTILLEUL, J. D. MILLER: Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. – Phytopathology **87**, 414–421, 1997.
- GILGENBERG-HARTUNG, A: Risiken durch Ährenfusariosen. **14**, 1999.
- HART, P., R. WARD: Efficacy of fungicides on *Fusarium* head blight severity and levels of vomitoxin. – In: Proceedings of National *Fusarium* Head Blight Forum, St. Paul, MN Eagan press, 40–41, 1997.
- HERMANN, W., E. KÜBLER, W. AUFHAMMER: Ährenbefall mit *Fusarium* und Toxingehalt im Korngut bei verschiedenen Wintergetreidearten. – Pfl. bauwiss. **2**, 97–107, 1998.
- HERMANN W., W. AUFHAMMER, E. KÜBLER, H.-P. KAUL: Auswirkungen von Ährenbefall mit *Fusarium graminearum* auf die Kornqualität von Winterweizen, Wintertriticale und Winterroggen. – Pfl. bauwiss. **3**, 82–87, 1999.
- HOFFER, J. N., A. G. JOHNSON, D. ATANASOFF: Corn-root rot and wheat scab. – J. Agric. Res. **13**, 611–612, 1918.
- HOLBERT, J. R., J. F. TROST, J. N. HOFFER: Wheat scabs as affected by systems of rotation. – Phytopathology **9**, 45–47, 1919.
- HUBER, D. M., R. D. WATSON: Crop residues, nitrogen, and plant disease. – Soil Sci. **100**, 302–308, 1965.
- HUI, Y., C. K. EVANS, E. K. KOLACZKOWSKI, M. R. DILL, C. J. MIROCHA, Y. HUI: Chemistry, physiology and role of deoxynivalenol in pathogenicity. – Bull. Inst. Comprehensive Agric. Sci., Kinki University, No. 5, 1–11, 1997.

- JENKINSON, P., D. W. PARRY: Isolation of *Fusarium* species from common broad leaved weeds and their pathogenicity to winter wheat. – Mycol. Res. **98**, 776–780, 1994.
- JONES, R. K., C. J. MIROCHA: Quality parameters in small grains from Minnesota affected by *Fusarium* head blight. – Pl. Dis. **83**, 506–511, 1999.
- JORDAN, V. W. L., J. A. HUTCHEON: Evaluation of an experimental fungicide against *Fusarium* disease of winter wheat in glasshouse and large pot experiments outside. – Report Long Ashton Research Station, 1989.
- KOEHLER, B., J. G. DICKSON, J. R. HOLBERT: Wheat scab and corn root rot caused by *Gibberella saubinetii* in relation to crop successions. – J. Agric. Res. **27**, 861–878, 1924.
- KRÜGER, W.: Mais Krankheiten + Schädlinge und deren Bekämpfung. – Saaten Union GmbH, Hannover, 1985.
- LBP (Bayer. Land. anst. Bodenkultur u. Pfl. bau): Ährenfusariosen des Getreides. 2. Auflage. – Freising, 1997.
- MARTIN, R. A., H. W. JOHNSTON: Effects and control of *Fusarium* diseases of cereal grains in the Atlantic provinces. Can. J. Pl. Path. **4**, 210–216, 1982.
- MARTIN, R. A., J. A. MACLEOD, C. CALDWELL: Influences of production inputs on incidence of infection by *Fusarium* species on cereal seed. – Pl. Dis. **75**, 784–788, 1991.
- MESTERHÁZY, Á.: Breeding for resistance to *Fusarium* head blight of wheat. – In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: Global status and future prospects, pp. 79–85, CIMMYT, Mexico, 1997a.
- MESTERHÁZY, Á.: Fungicide control of *Fusarium* scab and impact on toxin contamination. – In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: Global status and future prospects, pp. 120–124, CIMMYT, Mexico, 1997b.
- MIEDANER, T.: Entwicklung von Methoden zur Bestimmung der Fusarienresistenz in frühen Wachstumsstadien des Weizens. – Diss. Univ. Hohenheim, 1986.
- MIEDANER, T.: Review: Breeding wheat and rye for resistance to *Fusarium* diseases. – Pl. Breeding **116**, 201–220, 1997.
- MILLER, J. D., J. CULLEY, K. FRASER, S. HUBBARD, F. MELOCHE: Effect of tillage practice on *Fusarium* head blight of wheat. – Can. J. Pl. Path. **20**, 95–103, 1998.
- MILUS, E. A., C. E. PARSONS: Evaluation of folia fungicides for controlling *Fusarium* head blight of wheat. – Pl. Dis. **78**, 697–699, 1994.
- MIROCHA, C. J., H. K. ABBAS, C. E. WINDELS, W. XIE: Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. – Appl. Environm. Microbiol. **55**, 1315–1316, 1989.
- MÜLLER, H.-M., K. SCHWADORF: A survey of the natural occurrence of *Fusarium* toxins in wheat grown in a southwestern area of Germany. – Mycopathologia **121**, 115–121, 1993.
- OBST, A., J. LEPSCHY-V. GLEISSENTHAL, G. HUBER: Zur gezielten Bekämpfung der Ährenfusarien bei Weizen – Beobachtungen und Versuchsergebnisse aus Bayern. – Ges. Pfl. **44**, 40–47, 1992.
- OBST, A., P. GAMMEL: Fungizide gegen den Ährenparasiten *Fusarium graminearum*. – LBP (Bayer. Land. anst. Bodenkultur u. Pflanzenbau) **3**, 89–98, 2000.
- SAS Institute Inc.: SAS/STAT user's guide, version 6, fourth edition. – Cary, NC, USA, 1989.
- SCHWADORF, K., H.-M. MÜLLER: Determination of tricothecenes in cereals by gas chromatography with ion trap detection. – Chromatographia **32**, 137–142, 1991.
- SCOTT, N. A., C. V. COLE, E. T. ELLIOTT, S. A. HUFFMAN: Soil textural control on decomposition and soil organic matter dynamics. – Soil Sci. Soc. Am. J. **60**, 1102–1109, 1996.
- SEAMAN, W. L.: Epidemiology and control of mycotoxigenic fusaria on cereal grains. – Can. J. Pl. Path. **4**, 187–190, 1982.
- SMILEY R. W., R. J. COOK, R. I. PAPPENDICK: *Fusarium* foot rot of wheat and peas as influenced by soil applications of anhydrous ammonia and ammonia potassium azide solutions. – Phytopathology **62**, 86–91, 1972.
- SNIJEDERS, C. H. A.: Breeding for resistance to *Fusarium* in wheat and maize. – In: MILLER, J. D., H. L. THRENHOLM (eds.): Mycotoxins in grain-compounds other than aflatoxin, pp. 37–58. St Paul, MN Eagan press, 1994.
- SNYDER, W. C., S. M. NASH: Relative incidence of *Fusarium* pathogens of cereals in rotation plots at Rothamsted. – Trans. Br. Mycol. Soc. **51**, 417–425, 1968.

- STURZ, A. V., H. W. JOHNSTON: Characterization of *Fusarium* colonization of spring barley and wheat produced on stubble or fallow soil. – Can. J. Pl. Path. 7, 270–276, 1985.
- SUMMERELL, B. A., L. W. BURGESS, T. A. KLEIN, A. B. PATTISON: Stubble management and the site of penetration of wheat by *Fusarium graminearum* group 1. – Phytopathology 80, 877–879, 1990.
- SUTTON, J. C.: Epidemiology of wheat head blight and maize ear rot caused by *F. graminearum*. – Can. J. Pl. Path. 4, 195–209, 1982.
- TANAKA, T., S. YAMAMOTO, A. HASEGAWA, N. AOKI, J. R. BESLING, Y. SUGIURA: A survey of the natural occurrence of *Fusarium* mycotoxins, deoxynivalenol, nivalenol and zearalenone, in cereals harvested in the Netherlands. – Mycopathologia 110, 19–22, 1990.
- TEICH, A. H.: Less wheat scab with urea than with ammonium nitrate fertilizers. – Cereal Res. Commun. 15, 35–38, 1987.
- TEICH, A. H.: Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp. – In: CHELKOWSKI, J. (ed.): *Fusarium* mycotoxins, taxonomy and pathogenicity, pp. 269–282. Elsevier, Amsterdam, 1989.
- TEICH, A. H., K. NELSON: Survey of *Fusarium* head blight and possible effects of cultural practices in wheat fields in Lambton County in 1983. – Can. Pl. Dis. Surv. 64, 11–13, 1984.
- WANG, Y. Z.: Epidemiology and management of wheat scab in China. – In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. McNAB (eds.): *Fusarium* head scab: Global status and future prospects, pp. 97–105. CIMMYT, Mexico, 1997.
- WEINERT, J., G. A. WOLF: Erfahrungen zum Auftreten von Ährenfusariosen und Bekämpfungsstrategie im Weizen. – *Fusarium*-Symposium an der Universität Hohenheim, pp. 18–19. Stuttgart, 1999.
- WILCOXSON, R. D., R. H. BUSCH, E. A. OZMON: *Fusarium* head blight resistance in spring wheat cultivars. – Pl. Dis. 76, 658–661, 1992.
- WOLTZ, S. S., A. W. ENGELHARD: *Fusarium* wilt of chrysanthemum: Effect of nitrogen source and lime on disease development. – Phytopathology 63, 155–157, 1972.
- YI, C., H.-P. KAUL, E. KÜBLER, W. AUFHAMMER: Methodological problems in field investigations on *Fusarium graminearum* infection of wheat. – Z. Pfl. Krankh. Pl. Schutz 107, 583–593, 2000a.
- YI, C., H.-P. KAUL, E. KÜBLER, W. AUFHAMMER: Development of *Fusarium graminearum* on crop residues as affected by incorporation depth, fungicide application and nitrogen (submitted), 2000b.

## **Effects of residue management of *Fusarium graminearum* inoculated pre-crops on grain yield and grain quality of winter wheat**

Cuilin Yi, H.-P. Kaul, E. Kübler and W. Aufhammer

### **Summary**

*Fusarium* head blight of wheat (FHB), caused by *Fusarium graminearum*, has become a major problem world wide. *Fusarium* epidemics are considered to originate from inoculum associated with infested crop residues mainly of maize and wheat. The present study was conducted to determine the effect of management of *F. graminearum* infested pre-crop residues on grain yield and grain quality of winter wheat. In a 2-years factorial field experiment on the experimental station Ihinger Hof of the University of Hohenheim (480 m a.s.l., loam soil, 7.9°C, 690 mm), inoculated pre-crops of maize or spring wheat were harvested for silage with only the stubble remaining in the field or for grain by combine with the whole straw remaining. Subsequently, crop residues were left on the soil surface or ploughed under before sowing winter wheat. FHB of winter wheat test crops was assessed by observation of disease incidence and DON concentration. Grain yield as well as 1000-grain weight, protein content, sedimentation value and falling number were analysed. The infection level of winter wheat and the grain contamination with DON were similar after maize and spring wheat, either for silage or for grain. During the two experimental years, the *Fusarium* infection level of winter wheat was only moderate and ranged from 6-25% infected ears. FHB and grain contamination with DON were affected by residue management. The residue management did affect grain yield and grain quality, e. g. 1000-grain weight, protein content, sedimentation value and falling number slightly, but usually not to a meaningful degree. It might be more effective in epidemic years of *Fusarium*. In conclusion, at a moderate FHB infection level, the reduction of pre-crop residues after maize or spring wheat hardly influenced grain yield and grain quality of subsequent winter wheat, although it can be an effective cultural practice to reduce the FHB disease level and toxin contamination with DON in winter wheat to some degree. It will be necessary to investigate whether residue management can successfully increase the grain yield and grain quality of wheat grains at high infection levels of FHB during epidemic years.

**Key words:** winter wheat; yield; grain quality; *Fusarium* head blight; rotation; residue management

## 1 Introduction

*Fusarium* head blight (FHB) of wheat, caused by *Fusarium graminearum*, has become a major problem world wide. Epidemics cause extensive damages through losses in yield (30-70%, MIEDANER 1997), and consequently grains are contaminated with fungal metabolites, mainly deoxynivalenol (DON) (SUTTON 1982; MIROCHA et al. 1989; JENKINSON & PARRY 1994; SCOTT et al. 1996). Besides the toxicological aspect, reductions in grain quality, e. g. of protein content, sedimentation value and falling number, were observed with heavily infested crops (BECHTEL et al. 1985; MEYER et al. 1986; DEXTER et al. 1996; MIEDANER 1997; NIGHTINGALE et al. 1999). Some authors, however, found that the protein content even increased with infection level (MEYER et al. 1986; PAWELZIK et al. 1998).

*Fusarium* epidemics are generally considered to originate from inoculum associated with infested crop residues mainly of maize and wheat (SUTTON 1982; DILL-MACKY & JONES 2000; BECK & LEPSCHY 2000). RINTELEN (2000) assumes that the great increase of maize acreage since the sixties is responsible for the epidemic development of the disease. Maize and wheat grown in rotation leave abundant residues which are a primary source of inoculum. Ploughing to reduce *Fusarium*-inoculated crop residues on the soil surface may be a key to reduce the infection risk and protect grain yield and quality, since the efficient control of *F. graminearum* by fungicides is not possible, yet (FEHRMANN & DIEHL 1989; JORDAN & HUTCHEON 1989; MESTERHAZY 1997).

The choice of crop rotation and the management of crop residues can not only significantly affect disease levels and DON concentrations in grains (SNIJDERS 1994; WANG 1996; MILLER et al. 1998; DILL-MACKY & JONES 2000), but also influence the grain yield and the quality of bread wheat crops (ZENTNER et al. 1990; COX & SHELTON 1992; LÓPEZ-BELLIDO et al. 1998). BORCHI et al. (1995) found that the wheat yield from a maize-wheat-alfalfa rotation was about 25% above that from a monoculture and rotation significantly increased protein content. With no-till operation compared to conventional tillage a lower grain protein content and effects on other grain quality traits were observed, although the dough quality was not impaired (LÓPEZ-BELLIDO et al. 1998).

In the past decades, much research focused on studying the influence of crop rotation and residue management on FHB incidence and DON concentration in grains or the effect of FHB on grain yield and grain quality of wheat. But there are little data on effects of crop rotation in conjunction with management of infested residues on grain yield and grain quality of wheat. Different production aims – maize or spring wheat, both either harvested for silage or for grain use – shall result in greatly different amounts of crop residues left on the soil surface at harvest. The residues can be retained on the soil surface or ploughed under before sowing winter wheat crops. To these problem the present study was conducted in order to answer the following questions:

- Is the reduction of crop residues on the soil surface after maize or spring wheat pre-crops, which were inoculated with *Fusarium graminearum*, a suitable management practice to reduce the FHB disease level and DON contamination of subsequent winter wheat crops?
- How does the crop rotation in conjunction with the management of infested residues affect grain yield and grain quality traits of subsequent winter wheat?

## 2 Material and Methods

### 2.1 Experimental design

A factorial field experiment was conducted twice at the experimental station Ihinger Hof (480 m a. s. l., 7.9°C, 690 mm, loamy soil) of the University of Hohenheim in 1997-99 in a split-plot design with four replicates (Table 1, 2). Different pre-crops were arranged on main plots with treatments on sub-plots of 8 × 8 m size.

**Table 1.** Crop rotations in field experiments

Experiment	1997-1998		1998-1999	
1997	Maize*	Spring wheat*	Pre-experimental period	
1998	Winter wheat (Test crop)	Winter wheat (Test crop)	Maize*	Spring wheat*
1999	Post-experimental period		Winter wheat (Test crop)	Winter wheat (Test crop)

\*Maize and spring wheat were planted as pre-crops and inoculated with *F. graminearum* infected oat grains

**Table 2.** Factorial design of experiment

Experimental period	1997/98;1998/99
Pre-crop	Maize, cv. Helix; Spring wheat, cv. Hanno
Treatment (T)	T1: Pre-crops for silage, only stubble left on the soil surface; Non-inversion tillage (chisel plough) T2: Pre-crops for grain, straw residues ploughed into the soil; Inversion tillage (moldboard plough ~20 cm depth) T3: Pre-crops for grain, straw residues left on the soil surface; Tillage as with T1

Maize (cv. Helix) or spring wheat (cv. Hanno) were planted as pre-crops for winter wheat test crops in spring 1997 and 1998. The pre-crops were inoculated by *F. graminearum* infected oat grains. In both years, the inoculated pre-crop maize did



not show any infection symptoms at the stem base. On the contrary, the pre-crops of spring wheat were obviously infected with FHB. The disease incidence on spring wheat at BBCH 85 was on average 11% infected ears in 1997 and 53% in 1998 (YI et al. 2001). After harvest of the pre-crops, winter wheat (cv. Flair) was sown on October 28, 1997 and November 19, 1998, respectively. In parallel with the main wind direction, i. e. from west to east, test-plots were spaced 25 m apart with winter wheat crops in between in order to avoid inter-plot disease spread. Rectangular to the main wind direction, i. e. from north to south, 8 m wide isolation strips of tall growing winter rape were planted (YI et al. 2000, 2001).

The residues of the pre-crops were treated in different ways (T1-T3, Table 2). Pre-crops were either harvested for silage with only the stubble remaining in the field (T1) or they were harvested for grain by combine scattering the straw completely on the soil (T2, T3). Subsequently, the residues were either left on the soil surface (T1, T3) or ploughed under (T2) before sowing test crops of winter wheat. These test crops were fertilized at a rate of 160 kg N ha<sup>-1</sup> with calcium ammonium nitrate (CAN). In order to prevent a severe incidence of fungal diseases other than FHB, a fungicide (1.25 L ha<sup>-1</sup> Sportak Delta, a. i.: cyproconazol, prochloraz) not effective against *Fusarium* spp. was applied against foot rot, mildew and leaf spot during stem elongation.

## 2.2 Observations of winter wheat test crops in field and laboratory

In the field, the ear infection of winter wheat test crops was studied three times at medium (BBCH 75) and late milk ripeness (BBCH 77) as well as at medium dough ripeness (BBCH 85) from random samples of 50 ears per plot (Table 3). From these samples the infected ears were counted and means across three sampling dates were calculated to assess disease incidence (%). At full ripeness (BBCH 97), winter wheat was harvested per plot by combine and the grain yield was determined.

**Table 3.** Measured and calculated traits from test crop winter wheat stands and grain material

Trait	Dimension	Growth stage	Method
Disease incidence	Ear infection %	BBCH75-85	3 x 50 ears (YI et al., 2000a)
DON concentration	µg kg <sup>-1</sup> DM*	Harvested grains	GC-MS (SCHWADORF & MÜLLER, 1991)
Grain yield	dt DM* ha <sup>-1</sup>	Harvested grains	Plot harvest by combine
1000-grain weight	g DM*	Harvested grains	Counting and weighing
Protein content	%	Harvested grains	N x 5.7 (KLÜVER, 1994)
Sedimentation value	ml	Harvested grains	(KLÜVER, 1994)
Falling number	s	Harvested grains	(KLÜVER, 1994)

\*DM = dry matter

Under laboratory conditions, winter wheat grain samples from harvest were ground (1 mm sieve width). The ground material was thoroughly mixed and further subsampled for analysis of deoxynivalenol (DON) concentrations according to a method described by SCHWADORF & MÜLLER (1991). Furthermore, several grain quality traits, e. g. protein content, sedimentation value and falling number of test crop winter wheat were determined using standard methods (Table 3).

### 2.3 Statistical Analysis

At first, disease incidence was analyzed according to the factorial design of the field experiment using the General Linear Model (GLM) procedure of SAS (SAS 1989). Subsequently, all other traits were analyzed by covariance analysis with year, pre-crop and treatment as factors and disease incidence as a covariate. Corrected means, as adjusted for the effect of the covariate, are indicated as results. With significant effects ( $P < 0.05$ ), Least Significant Differences ( $LSD_{0.05}$ ) were calculated for the separation of means.

## 3 Results

### 3.1 Disease incidence

FHB infections of the test crop winter wheat occurred obviously in both years, but the infection level was generally moderate and did not exceed 25% (Table 4). The infection was slightly higher in 1999 compared with 1998, but there were no consistent differences between pre-crops. When crop residues were ploughed under (T2), the infection level was always lower than with residues on the soil surface, either after silage harvest (T1) or after grain harvest (T3). Especially the infection level after pre-crops of maize for silage in 1998 was high.

**Table 4.** Disease incidence of test crop winter wheat (means from 3 sampling dates) dependent on year, pre-crop and treatment

Treatments	Disease incidence (%)			
	1998		1999	
	Maize	Spring wheat	Maize	Spring wheat
T1 (silage, no-till)	25	9	16	20
T2 (grain harvest, plough)	6	6	12	9
T3 (grain harvest, no-till)	10	8	19	14
$LSD_{0.05}$	12			

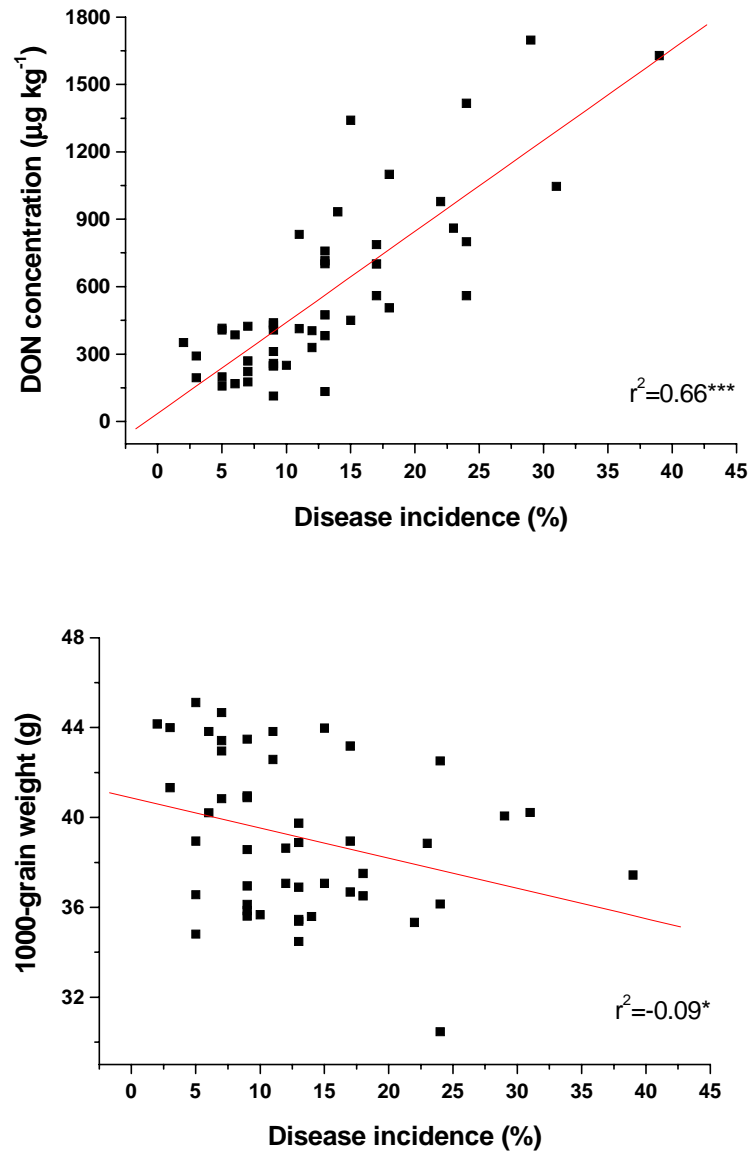
**Table 5.** Results of covariance analyses (F values) of different traits of test crop winter wheat (2 years  $\times$  2 pre-crops  $\times$  3 treatments  $\times$  4 replicates)

Source of variation	DON concentration ( $\mu\text{g kg}^{-1} \text{DM}^{\text{a}}$ )	Grain yield ( $\text{dt DM}^{\text{a}} \text{ha}^{-1}$ )	1000-grain weight ( $\text{g DM}^{\text{a}}$ )	Protein content (%)	Sedimentation value (ml)	Falling number (s)
Year (Y)	0.50	259.35***	38.60**	933.75***	1204.64***	246.67***
Pre-crop (P)	4.70	0.42	6.74*	0.17	0.05	1.76
Treatment (T)	3.81*	6.47**	0.53	1.84	1.27	0.07
Y $\times$ P	16.03**	0.50	1.27	0.21	1.15	0.91
Y $\times$ T	0.95	3.35*	0.52	0.49	1.13	0.77
P $\times$ T	0.98	1.22	1.06	0.02	4.73*	0.72
Y $\times$ P $\times$ T	6.47**	1.51	1.63	0.98	2.14	1.25
Disease incidence (%)	5.38*	3.44	7.25**	0.82	0.18	0.34

\*, \*\*, \*\*\* significant effects at  $P < 0.05, 0.01, 0.001$ ; <sup>a</sup>DM = dry matter.

### 3.2 Effects of disease incidence on DON concentration, grain yield and quality

The covariance analyses revealed significant effects of disease incidence on DON concentration and 1000-grain weight of the test crop winter wheat, but no significant effects on grain yield or other grain quality traits (Table 5). Fig. 1 shows that the relationship between disease incidence and DON concentration was comparatively strong but 1000-grain weight was only slightly impaired with increasing disease incidence.



**Fig. 1.** Relationship between disease incidence (means from 3 sampling dates) and DON concentration as well as 1000-grain weight of harvested grains.  
N=48 (2 years × 2 pre-crops × 3 treatments × 4 replicates)

### 3.3 Effects of pre-crops and residue treatments on DON concentration, grain yield and grain quality

The DON concentrations of test crop winter wheat, as adjusted for the effect of disease incidence, showed comparatively low levels when crop residues were ploughed under (T2, Table 6). In 1998, the toxin levels were higher after maize than after spring wheat with an outstanding result after maize for silage. On contrast, in 1999 higher DON concentrations were observed after spring wheat.

Grain yield of winter wheat was substantially higher in 1998 compared with 1999. But there were no obvious differences referring to the pre-crops maize and spring wheat. Silage use of the pre-crops (T1) improved yield, but differences to the other residue treatments were only moderate.

**Table 6.** DON concentration, grain yield and 1000-grain weight of harvested grains of winter wheat dependent on year, pre-crop and treatment (means are adjusted for the effect of disease incidence)

Treatments	Adjusted means											
	DON concentration ( $\mu\text{g kg}^{-1}$ )				Grain yield (dt DM* ha <sup>-1</sup> )				1000-grain weight (g DM*)			
	1998		1999		1998		1999		1998		1999	
	Maize	Spring wheat	Maize	Spring wheat	Maize	Spring wheat	Maize	Spring wheat	Maize	Spring wheat	Maize	Spring wheat
T1	1155	382	487	807	68.3	68.1	55.1	55.1	43.5	40.2	37.2	37.3
T2	473	377	536	336	61.9	61.6	50.1	50.5	43.4	39.8	36.6	35.6
T3	619	385	496	667	56.6	57.1	57.5	47.8	42.8	39.9	38.3	35.0
LSD <sub>0.05</sub>	389				n.s.				n.s.			

\*DM = dry matter

**Table 7.** Sedimentation value of harvested grains of winter wheat dependent on pre-crop and treatment (means are adjusted for the effect of disease incidence)

Treatments	Adjusted means	
	Sedimentation value (ml) of winter wheat	
	Maize	Spring wheat
T1 (silage, no-till)	30.61	32.94
T2 (grain harvest, plough)	34.51	32.32
T3 (grain harvest, no-till)	32.69	32.07
LSD <sub>0.05</sub>	2.37	

In 1998, 1000-grain weight of winter wheat was higher compared with 1999. Especially under the environmental conditions of 1998, winter wheat kernels after maize were heavier than those after wheat. Residue management did not affect 1000-grain weight of winter wheat.

Grain protein content and sedimentation value of winter wheat were substantially higher in 1999 (means: 14.0% protein, 38.7 ml sedimentation value) compared with 1998 (11.3% protein, 26.3 ml sedimentation value). But the protein content was not affected either by pre-crops or by treatments (Table 5). The sedimentation value of winter wheat after maize, however, did react on residue treatments. The lowest

sedimentation volume occurred after pre-crops for silage (T1) and the highest after incorporation of pre-crop residues (T2). After spring wheat, the sedimentation value of the subsequent winter wheat was not affected by residue treatments.

The falling number of winter wheat was substantially influenced by year. It was higher in 1998 (358 s) compared with 1999 (279 s), but pre-crops and residue treatments did not show any effect (Table 5).

#### 4 Discussion

In the past decades, much research was conducted on the effects of crop rotation and residue management on *Fusarium* head blight (FHB) as well as on the effects of FHB on grain yield and grain quality, respectively (TEICH & NELSON 1984; ZENTNER et al. 1990; COX & SHELTON 1992; SNIJDERS 1994; BORGHI et al. 1995; WANG 1996; LÓPEZ-BELLIDO et al. 1998; MILLER et al. 1998; DILL-MACKY & JONES 2000). Yet studies on direct effects of crop rotation in conjunction with management of infested residues on grain yield and grain quality of wheat are rare. Therefore, the concern of the present study was whether we could successfully increase the grain yield and grain quality of winter wheat through reducing the crop residues from *Fusarium* inoculated pre-crops on the soil surface due to silage use or by ploughing under the residues of combined crops. Because wheat following maize in a rotation system is presumably more prone to severe FHB infections than wheat after wheat (HOFFER et al. 1918; KOEHLER et al. 1924; SNYDER & NASH 1968; SEAMAN 1982; TEICH & NELSON 1984; RINTELEN 2000), maize and spring wheat were chosen as pre-crops.

The FHB infection level of winter wheat was only moderate during the two experimental years and ranged from 6-25% (Table 4). It was higher in 1999 than in 1998, presumably because the climate conditions during the periods of investigation in 1999 were more favourable for infection of *F. graminearum* (Yi et al. 2001). Although the pre-crop maize did not show any infection symptoms of *Fusarium* foot rot at the stem base, the ears of spring wheat were clearly infected with *F. graminearum*. The main reasons for the difference in inoculation success were discussed in a previous paper (Yi et al. 2001). Moreover, there were no significant differences in infection level of winter wheat observed between the pre-crops maize or spring wheat. Assumedly the comparatively low infection level of winter wheat after maize in this study was due to the missing infection of maize stems. While the different pre-crops – maize or spring wheat, either for silage or for grain – showed hardly any effect, the infection level was affected by residue management, mainly by soil tillage.

In order to identify the effects of year, pre-crop and residue treatment on DON concentration, grain yield and quality of test crop winter wheat, covariance analyses were conducted to exclude the interference of disease incidence. The adjusted

means of DON concentrations from harvested grains of winter wheat reflected the moderate infection level, but a threshold value of 1 mg DON kg<sup>-1</sup>, which is expected for food grain in Europe, was occasionally exceeded. Grain contamination with DON was affected by residue management – ploughing the crop residues under (T2) could obviously reduce DON concentration in infected winter wheat grains in comparison with residues left on the soil surface, either after silage (T1) or after grain harvest (T3). Though the maize was not visibly infected, residue management showed effects on infection level and DON concentration of the test crop winter wheat. If the maize would be stronger infected, residue management might be even more effective.

Grain yield and 1000-grain weight of the test crop winter wheat were significantly higher in 1998 compared with 1999. Presumably an important reason was the stronger infection of winter wheat in 1999 compared to 1998. The silage use of pre-crops improved winter wheat yield moderately. The maize pre-crop increased 1000-grain weight by 3.9-8.2% in 1999 and 1998, compared with the spring wheat pre-crop. However, the reduction of pre-crop residues could not substantially increase the 1000-grain weight of the subsequent winter wheat. The sedimentation value was strongly affected by year. Since sedimentation volumes within a relatively large range from 30-45 ml indicate a good baking quality, the observed differences of 4-5 ml are not very important. The protein content and the falling number differed significantly between years. But the slight differences in protein content and falling number dependent on pre-crops and management of the subsequent infested residues were not important under applied aspects. In agreement with SEITZ et al. (1986) and HERMANN et al. (1999), cultural practices and crop husbandry affected grain yield and grain quality slightly but usually not to a meaningful degree. They might be more effective in epidemic years of *Fusarium*. The potential inoculum for FHB is reported to be mainly ascospores produced on pre-crop residues that have remained on the soil surface (KHÖGA & SUTTON 1986). Practices for suppressing initial inoculum, especially rotation of wheat and maize with non-host crops and ploughing infested residues, have long been recommended for managing FHB (MARTIN & JOHNSTON 1982; MILLER et al. 1998; DILL-MACKY & JONES 2000). But up-to-date we have still no evidence that reducing the crop residues from *Fusarium* inoculated pre-crops on the soil surface either by silage use or by ploughing under the residues of combined crops can significantly increase the grain yield and grain quality of winter wheat.

In conclusion, at a moderate FHB infection level, the reduction of pre-crop residues after maize or spring wheat hardly influenced grain yield and grain quality of subsequent winter wheat, although it can be an effective cultural practice to reduce the FHB disease level and toxin contamination with DON in winter wheat to some degree. It will be necessary to investigate whether residue management can

successfully increase the grain yield and grain quality of wheat grains at high infection levels of FHB during epidemic years.

## Acknowledgements

The authors gratefully acknowledge the technical support of the grain quality analyses by the experimental station Ihinger Hof, namely by Dr. G. Renz, H. Kärcher, M. Zahner and H. Grözinger. The authors also thank for the technical support of the DON analyses by the State Institute for Agricultural Chemistry at the University of Hohenheim, namely by Prof. Dr. H. Schenkel, Dr. B. Eckstein, Dr. K. Schwadorf and Mrs. A. Eschle.

## Literature

- BECHTEL, D. B., L. A. KALEIKAU, L. A. GAINES, L. M. SEITZ: The effects of *Fusarium graminearum* infection on wheat kernels. - Cereal Chemistry. **62**, 191-197, 1985.
- BECK, R., J. LEPSCHY: Ergebnisse aus dem Fusarium-Monitoring 1989-1999 - Einfluss der produktionstechnischen Faktoren Fruchtfolge und Bodenbearbeitung. - LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau) **3**, 39-47, 2000.
- BORCHI, B., G. GIORDANI, M. CORBELLINI, P. VACCINO, M. GUERMANDI, G. TODERI: Influence of crop rotation, manure and fertilizers on bread making quality of wheat (*Triticum aestivum* L.). - Eur. J. Agron. **4**, 37-45, 1995.
- COX, D. J., D. R. SHELTON: Genotype-by-tillage interactions in hard red winter wheat quality evaluation. - Agron. J. **84**, 627-630, 1992.
- DEXTER, J. E., R. M. CLEAR, K. R. PRESTON: *Fusarium* head blight: Effect on the milling and baking of some Canadian wheats. - Cereal Chem. **73**, 695-701, 1996.
- DILL-MACKY, R., R. K. JONES: The effect of previous crops residues and tillage on *Fusarium* head blight of wheat. - Plant Disease **84**, 71-76, 2000.
- FEHRMANN, H., T. DIEHL: Partielle Taubährigkeit chemisch behandeln. - Pflanzenschutz-Praxis. **2**, 29-31, 1989.
- HERMANN W., W. AUFHAMMER, E. KÜBLER, H.-P. KAUL: Auswirkungen von Ährenbefall mit *Fusarium graminearum* auf die Kornqualität von Winterweizen, Wintertriticale und Winterroggen. - Pflanzenbauwiss. **3**, 82-87, 1999.
- HOFFER, J. N., A. G. JOHNSON, D. ATANASOFF: Corn-root rot and wheat scab. - J. Agric. Res. **13**, 611-612, 1918.
- JENKINSON, P., D. W. PARRY: Isolation of *Fusarium* species from common broad leaved weeds and their pathogenicity to winter wheat. - Mycol. Res. **98**, 776-780, 1994.



- JORDAN, V. W. L., J. A. HUTCHEON: Evaluation of an experimental fungicide against *Fusarium* disease of winter wheat in glasshouse and large pot experiments outside. - Report Long Ashton Research Station, 1989.
- KHONGA, E. B., J. C. SUTTON: Survival and inoculum production of *Gibberella zeae* in wheat and maize residues. - Can. J. Plant Pathol. **8**, 351, 1986.
- KLÜVER, M.: - in KLÜVER, M. (ed), Standard Methoden für Getreide, Mehl und Brot. 7, Überarbeitete und erweiterte Auflage, Arbeitsgemeinschaft Getreideforschung e.V., Verlag Moritz Schäfer, Detmold, 1994.
- KOEHLER, B., J. G. DICKSON, J. R. HOLBERT: Wheat scab and corn root rot caused by *Gibberella saubinetii* in relation to crop successions. - J. Agric. Res. **27**, 861-878, 1924.
- LÓPEZ-BELLIDO, L., M. FUENTES, J. E. CASTILLO, F. J. LÓPEZ-CARRIDO: Effects of tillage, crop rotation and nitrogen fertilization on wheat-grain quality grown under rainfed Mediterranean conditions. - Field Crops Research. **57**, 265-276, 1998.
- MARTIN, R. A., H. W. JOHNSTON: Effects and control of *Fusarium* diseases of cereal grains in the Atlantic provinces. - Can. J. Plant Pathol. **4**, 210-216, 1982.
- MESTERHAZY, A.: Fungicide control of *Fusarium* scab and impact on toxin contamination. - In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. MCNAB (eds), *Fusarium* Head Scab: Global status and future prospects, CIMMYT, Mexico, 120-124, 1997.
- MEYER, D., D. WEIPERT, H. MIELKE: Beeinflussung der Qualität von Weizen durch den Befall mit *Fusarium culmorum*. - Getreide, Mehl Brot. **40**, 35-39, 1986.
- MIEDANER, T.: Review: Breeding wheat and rye for resistance to *Fusarium* diseases. - Plant Breeding **116**, 201-220, 1997.
- MILLER, J. D., J. CULLEY, K. FRASER, S. HUBBARD, F. MELOCHE: Effect of tillage practice on *Fusarium* head blight of wheat. - Can. J. Plant Pathol. **20**, 95-103, 1998.
- MIROCHA, C. J., H. K. ABBAS, C. E. WINDELS, W. XIE: Variation in dextynivalenol, 15-acetydeoxynivalenol, 3-acetydeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolated. - Applied and Environmental Microbiology. **55**, 1315-1316, 1989.
- NIGHTINGALE, M. J., B. A. MARCHYLO, R. M. CLEAR, J. E. DEXTER, K. R. PRESTON: *Fusarium* Head Blight: Effect of fungal proteases on wheat storage proteins. - Cereal Chem. **76**, 150-158, 1999.
- PAWELZIK, E., H. H. PERMADY, J. WEINERT, G. A. WOLF: Einfluß von *Fusarium* auf die Qualität von Winterweizen. - Kongreßband, 299-302, 1998.
- RINTELEN, J.: Ist das starke auftreten von *Gibberella zeae* (*Fusarium graminearum*) an Getreideähren auf die Zunahme des Maisanbaus zurückzuführen? - LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau). **3**, 11-14, 2000.

- SAS Institute Inc.: SAS/STAT user's guide, version 6, fourth edition, - Cary, NC, USA, 1989.
- SCHWADORF, K., H.-M. MÜLLER: Determination of trichothecenes in cereals by gas chromatography with ion trap detection. - *Chromatographia*. **32**, 137-142, 1991.
- SCOTT, N. A., C. V. COLE, E. T. ELLIOTT, S. A. HUFFMAN: Soil textural control on decomposition and soil organic matter dynamics. - *Soil Sci. Soc.* **60**, 1102-1109, 1996.
- SEAMAN, W. L.: Epidemiology and control of mycotoxigenic fusaria on cereal grains. - *Can. J. Plant Path.* **4**, 187-190, 1982.
- SEITZ, L. M., W. D. EUSTACE, H. E. MOHR, M. D. SHOGREN, W. T. YAMAZAKI: Cleaning, milling, and baking tests with hard red winter wheat containing deoxynivalenol. - *Cereal Chem.* **63**, 146, 1986.
- SNIJDERS, C. H. A.: Breeding for resistance to *Fusarium* in wheat and maize. - In: MILLER, J. D., H. L. THRENHOLM (eds), *Mycotoxins in grain-compounds other than aflatoxin*, St Paul, MN Eagan press, pp. 37-58, 1994.
- SNYDER, W. C., S. M. NASH: Relative incidence of *Fusarium* pathogens of cereals in rotation plots at Rothamsted. - *Trans. Br. Mycol. Soc.* **51**, 417-425, 1968.
- SUTTON, J. C.: Epidemiology of wheat head blight and maize ear rot caused by *F. graminearum*. - *Can. J. Plant Path.* **4**, 195-209, 1982.
- TEICH, A. H., K. NELSON: Survey of *Fusarium* head blight and possible effects of cultural practices in wheat fields in Lambton County in 1983. - *Can. Plant dis. Surv.* **64**, 11-13, 1984.
- WANG, Y. Z.: Epidemiology and management of wheat scab in China. - In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. MCNAB (eds), *Fusarium Head Scab: Global status and future prospects*, CIMMYT, Mexico, 97-105, 1996.
- YI, C., H.-P. KAUL, E. KÜBLER, W. AUFHAMMER: Methodological problems in field investigations on *Fusarium graminearum* infection of wheat. - *Plant Diseases and Protection* **107**, 583-593, 2000.
- YI, C., H.-P. KAUL, E. KÜBLER, W. AUFHAMMER: Head blight (*Fusarium graminearum*) and deoxynivalenol concentration in winter wheat as affected by pre-crop, soil tillage and nitrogen fertilization. - *Plant Diseases and Protection* **108**, 217-230, 2001.
- ZENTNER, R. P., K. E. BOWREN, W. EDWARDS, C. A. CAMPBELL: Effects of crop rotations and fertilization on yields and quality of spring wheat grown on a black Chernozem in north central Saskatchewan. - *Can. J. plant Sci.* **70**, 383-397, 1990.

## **E. Summary of main results**

### **1. Isolation-strip field experiment**

The 2-year field experiment with wheat showed that the isolation-strips are a suitable measure to limit the spread of *F. graminearum* spores in plot experiments in the field with artificially inoculated plots. 2 m broad isolation strips of winter rape crops growing 50 cm higher than the wheat crops reduced disease incidence on test plots by more than 50% in most cases. A further increase in isolation strip width (4 or 8 m) improved the isolation effect, but the differences between isolation strip widths were comparatively small. Even with 8 m wide strips the infection in test plots was not completely eliminated.

### **2. Residue management greenhouse tests**

The results of greenhouse tests with both residue types (maize and spring wheat) showed that deeper residue incorporation slightly reduced the decomposition process but effectively reduced the population level of *F. graminearum*. The application of nitrolime did not affect the decomposition process but reduced the population level of *F. graminearum*. Especially the nitrolime can help to reduce the populations of *F. graminearum* on the residues without detrimental effects on the decomposition process. On the contrary, fertilization with calcium ammonium nitrate sometimes even reduced residue decomposition but promoted the population level of *F. graminearum*. Soaking the residues in a fungicide preparation impaired the decomposition process substantially and eliminated *F. graminearum* on the residues completely.

### **3. Residue management field experiment**

During a 2-year main field experiment, the inoculated pre-crop maize did not show any infection symptoms of *Fusarium* foot rot at the stem base, but the ears of the inoculated spring wheat were clearly infected with *F. graminearum* on an average of 11% in 1997 and 53% in 1998. Moreover, in both years there were no significant differences in the infection levels of the test crop winter wheat following after the pre-crops maize or wheat. The infection level of winter wheat was especially high after maize for silage use in 1998. In general, FHB incidence in winter wheat reached only moderate levels in these two experimental years (6-25%). The infection level of the test crop winter wheat was higher in 1999 than that of the test crop in 1998, because the climate conditions during the periods of investigation in 1999 were more favourable for infection of *F. graminearum* than in 1998. The reductions of FHB incidence due to ploughing or nitrolime application were 27-32% or 31-59% compared with residues remaining on the surface or CAN

(calcium ammonium nitrate) fertilization, respectively. Grain contamination with DON was also affected by the residue management. But at a moderate FHB infection level, the reduction of pre-crop residues after maize or spring wheat hardly influenced grain yield and grain quality of subsequent winter wheat, although it can be an effective cultural practice to reduce the FHB disease level and the toxin contamination with DON in winter wheat to some degree.

## F. General discussion

*F. graminearum* is able to spread spores across large distances within crops. For plot experiments in the field with artificial inoculation, simulating the natural way of infection, it has to be considered whether individual treatments can be efficiently separated from each other by isolation strips. The results of the isolation-strips field experiment showed that the isolation-strips did significantly reduce the infection of wheat on test plots. The isolation effect improved slightly with increasing strip width. But it has to be emphasised that the spread of inoculum from plots inoculated with infected oat grains on the soil cannot be reduced to a negligible level even by isolation-strips of 8 m width. According to BECK et al. (1997) distances of up to 30 m from the source can be passed, at least without any isolation-strips inserted. Up to now, no other experimental data are available to recommend more suitable crops or cultivars for the isolation purpose. Species like hemp or sunflower grow much taller than winter rape, but because they develop too slowly in spring compared with wheat crops, at least under our climatic conditions, they are not suitable for segregation. In our residue management field experiment with *F. graminearum*, the isolation widths between adjacent sub-plots of 25 m of winter wheat in parallel with the main wind direction and 8 m of winter rape rectangular to the main wind direction should have reduced inter-plot disease spread to a high degree, although it could not be completely eliminated (FERNANDO et al. 1997). Under these conditions, the statistical significance of effects on the infection level due to factors of crop husbandry can only be expected from experiments with a properly randomized design and a sufficient number of replicates.

Contrasting to artificial inoculations by spraying spore suspensions on the ears, we inoculated wheat with *F. graminearum* by infected oat grains during early stages of crop development simulating the natural infection conditions. The infection starts from residues of preceding crops on the soil surface and it is influenced by characteristics of the crop canopy and wind effects. The wind dispersal of ascospores, however, causes severe problems in field plot experiments due to not intended contamination of non-inoculated plots. Of course, spray inoculation allows for a much better control of the infection, but this method excludes the natural infection process starting at the soil surface.

In order to suppress *F. graminearum* development on residues, we can either strive for a direct control by a fungicide or we can try to influence the residue decomposition by residue placement and nitrogen application. Our results showed that the soaking of the residues in a fungicide solution (a. i.: tebuconazole) did not only substantially decrease the decompositions of crop residues, but also stopped fungal growth and development completely. Obviously, fungicides with tebuconazole are effective against *F. graminearum* as well as against other decomposing micro-organisms (FEHRMANN & DIEHL 1989; JORDAN & HUTCHEON

1989). In view of our results from soaking residues in a fungicide preparation, we might conclude for agronomic practice, that it would be useful to suppress *F. graminearum* development by treating residues shortly after harvest with relative high concentrations of a fungicide. But our results stand opposite to results of MOLLENHAUER et al. (1999), who found that the spray application of fungicides on cereals has no effect on the rate of straw decomposition. Presumably, this contradiction can be explained by the large difference in the concentrations applied by spraying on standing crops or by soaking the straw in a fungicide solution.

Incorporation depth has a significant influence on crop residue decomposition and the populations of *F. graminearum* on the residues. The decomposition of residues and the population of *F. graminearum* on the residues, namely the number of colony forming units (CFU) and the conidiospore density decreased with increasing incorporation depth of the residues. After 90 days of greenhouse tests, the burial of crop residues at 10 cm or at 15 cm reduced the number of colonies by 16-28% or 39-42%, respectively, compared with the surface incorporation at 5 cm.

The application of nitrolime reduced the population level of *F. graminearum* on crop residues, but the applied N rate was comparatively high. Presumably, the main reason is that metabolites of nitrolime formed in the soil, especially cyanamide, have mycostatic effects. Additionally, urea is formed which was shown to reduce *Fusarium* infection compared with ammonium nitrate fertilizer (TEICH 1987). On the contrary, fertilization with calcium ammonium nitrate (CAN) promoted the *Fusarium* populations compared to the control (no nitrogen application).

Conservation tillage systems, which maintain crop residues on the soil surface, are becoming more widely used. The residues serve as ground cover to reduce soil erosion and act as a sink-source for plant nutrients (HUBBARD & JORDAN 1996), but they are also involved in the propagation of *Fusarium*. Agronomic practices such as tillage, residue management, and crop rotation all play important roles in determining the risk of this disease, by influencing the type, amount and location of inoculum. COOK (1981) concluded from a literature review that maximum burial of host crop residues is probably useful in *Fusarium* control since inoculum is released only from the infested residue lying on the soil surface.

During our residue management field experiments, pre-crops of maize or spring wheat in rotations with winter wheat were inoculated by *F. graminearum* infected oat grains. The intention was to simulate the natural inoculum source of crop residues and the infection process originating from these residues. But in both years, the pre-crop maize did not show any infection symptoms of *Fusarium* foot rot at the stem base. On the contrary, the ears of spring wheat were clearly infected with *F. graminearum*. The missing infection of maize may have been due to the smaller amount of inoculum that had been applied to maize ( $15 \text{ g m}^{-2}$ ) as compared to wheat ( $25 \text{ g m}^{-2}$ ) or to the different dates of inoculation. Maybe the inoculation of maize

before flowering was too early for stem infection. Even without infection of living maize plants, it is reasonable to assume that *Fusarium* populations, which are well known as crop residue decomposers, are able to grow and develop on dead maize straw. Beyond these arguments, the artificial inoculation of maize in fields without a soil borne infection potential might be a methodical problem that deserves further investigation.

The infection level of the winter wheat test crops did not exceed 25%. Also, the deoxynivalenol (DON) concentrations in the grain material of winter wheat were on a moderate level compared with the concentrations which are observed after spray inoculation of ears with spore suspensions at heading of crops (AUFHAMMER et al. 2000). However, a threshold value of 1 mg DON kg<sup>-1</sup> grain, which is expected for food grain in Europe, was occasionally exceeded. Obviously, DON concentrations at that level are easily obtained under natural infection conditions (MÜLLER & SCHWADORF 1993; JONES & MIROCHA 1999).

The infection of winter wheat crops with *F. graminearum* and the consequences for the grain contamination with DON are affected by soil tillage and nitrogen fertilization, but the different pre-crops - maize or spring wheat, either for silage or for grain - showed hardly any effect. This is in contradiction to other investigations which suggest that wheat following maize in a rotation system is more prone to severe FHB infections than wheat after wheat (HOFFER et al. 1918; HOLBERT et al. 1919; KOEHLER et al. 1924; SNYDER & NASH 1968; SEAMAN 1982; SUTTON 1982; TEICH & NELSON 1984). Maybe the comparatively low infection levels after maize in our experiments were due to the missing infection of maize stems. On the other hand, although the maize was not visibly infected, there were no differences in infection levels of the test crop winter wheat observed between the pre-crops maize or wheat and in one year the DON concentration in wheat grain was even higher after maize than after wheat for silage.

It cannot be decided confidently, whether the primary source of inoculum were really the crop residues or the oat grains. The oat grains were still visibly lying on the soil surface at harvest of the pre-crops. The considerable infection level after maize suggests an eventual role of the inoculation material of oat grains. Moreover, the slightly higher infection level after silage use of the pre-crops compared with grain use may have resulted from an easier propagation of *Fusarium* spores without large amounts of straw on the soil surface. There were different possible sources of inoculum, including the oat grains, the wheat chaff or the maize stubble, that might have been covered by this hardly infected straw material. Additionally, the harvest residues of pre-crops after silage lie on the soil surface for a longer period than those after grain harvest. Other authors report a higher infection risk after maize for grain compared with silage maize, which is presumably due to much stronger

infected maize crops with heavier infested straw residues (OBST 1997; RINTELEN 1997).

Crop residues in no-till plots can provide a significant source of *Fusarium* inoculum (MILLER et al. 1998). Comparatively small amounts of residues after silage harvest of maize or spring wheat were already sufficient for a high level of infection. *F. graminearum* can persist on residues under both till and no-till conditions and infections can occur in subsequent years. However, ploughing under of residues before sowing a subsequent wheat crop reduced the prevalence of FHB in the following year and the grain contamination with DON. Ploughing reduced FHB incidence compared with surface residues by 27-32% in 1998 and 1999, respectively.

SUTTON & VYN (1990) suggested that increasing concentrations of organic acids or other antifungal substances may act also to suppress the population of *Fusarium* to grow and develop on crop residues, thus reduce the FHB incidence of subsequent crops. Liming reduced the severity of *Fusarium* wilt (*F. oxysporum*) and nitrate-nitrogen applied in addition to calcium hydroxide decreased *Fusarium* wilt even more (WOLTZ & ENGELHARD 1972). The incidence and severity of *Fusarium* root-rot diseases of winter wheat was higher after fertilization with ammonium ( $\text{NH}_4$ ) compared with nitrate ( $\text{NO}_3$ ), and wheat fertilized with urea ( $\text{CO}(\text{NH}_2)_2$ ) showed less FHB symptoms than wheat fertilized with ammonium nitrate (HUBER & WATSON 1965; SMILEY et al. 1972; TEICH 1987; MARTIN et al. 1991). Moreover, the importance of the amount of nitrogen and the type of nitrogen fertilizer for the populations of *Fusarium* spp. and disease incidence were stressed by many authors (HUBER & WATSON 1965; SMILEY et al. 1972; WOLTZ & ENGELHARD 1972; TEICH 1987, 1989; MARTIN et al. 1991; AUFHAMMER et al. 2000). In our present study, nitrolime showed a fungistatic effect in the field and pot experiments. It reduced FHB incidence of test crop winter wheat in field experiment compared with CAN fertilization by 31-59% in 1999 and 1998, respectively.

In the past decades, much research was conducted on the effects of crop rotation and residue management on FHB as well as on the effects of FHB on grain yield and grain quality, respectively (TEICH & NELSON 1984; ZENTNER et al. 1990; COX & SHELTON 1992; SNIJDERS 1994; BORCHI et al. 1995; WANG 1996; LÓPEZ-BELLIDO et al. 1998; MILLER et al. 1998; DILL-MACKY & JONES 2000). But studies on direct effects of crop rotation in conjunction with management of infested residues on grain yield and grain quality of wheat are rare. Therefore, the effect of residue management on grain yield and grain quality of winter wheat was also an important aspect in the present study. The results showed that the grain contamination with DON was affected by residue management – ploughing the crop residues under obviously reduced DON concentration in infected winter wheat grains in comparison with residues left on the soil surface, either after silage or after



grain harvest. Though the maize was not visibly infected, residue management showed effects on infection level and DON concentration of the test crop winter wheat. If the maize would be stronger infected, residue management might be even more effective.

The silage use of pre-crops improved winter wheat yield moderately. The maize pre-crop increased the 1000-grain weight by 4-8% in 1998 and 1999, compared with spring wheat. However, the reduction of pre-crop residues could not substantially increase the 1000-grain weight of the subsequent winter wheat. The sedimentation values were within a relatively large range from 30-45 ml, indicating a good baking quality. Slight differences in protein content and falling number dependent on pre-crops and management of the subsequent infested residues were not important under applied aspects. In agreement with SEITZ et al. (1986) and HERMANN et al. (1999), cultural practices and crop husbandry affected grain yield and grain quality slightly but usually not to a meaningful degree. They might be more effective in epidemic years of *Fusarium*.

The potential inoculum FHB were reported to be mainly ascospores produced on pre-crop residues that have remained on the soil surface (KHÖGA & SUTTON 1986). Practices for suppressing initial inoculum, especially rotations of wheat and maize with non-host crops and ploughing under infested residues, have long been recommended for managing FHB (MARTIN & JOHNSTON 1982; MILLER et al. 1998; DILL-MACKY & JONES 2000). But up-to-date we have still no evidence that reducing the crop residues from *Fusarium* inoculated pre-crops on the soil surface either by silage use or by ploughing under the residues of combined crops can significantly increase the grain yield and grain quality of winter wheat. Therefore, it seems necessary to investigate whether residue management can successfully increase the grain yield and grain quality of wheat grains at high infection levels of FHB during epidemic years.

## G. Summary/Zusammenfassung

### Summary

#### 1. Introduction

*Fusarium* head blight (FHB), caused by *F. graminearum*, has become one of the most destructive cereal diseases world wide. Epidemics cause extensive damages through losses in grain yield, and grains consequently are contaminated with fungal metabolites, mainly deoxynivalenol (DON). Furthermore, reductions in technological grain quality were observed with heavily infested crops. *Fusarium* epidemics are considered to originate from inoculum associated with infested crop residues mainly of maize and wheat. The main inoculum is reported to be ascospores produced on crop residues on the soil surface. The management of *Fusarium*-infested crop residues of maize or spring wheat may be a key to reduce the infection risk and protect grain yield and grain quality.

#### 2. Problem definition

The present study combines field experiments and greenhouse tests in order to answer the following questions: Is it possible to suspend the dispersal of *F. graminearum* between field plots of winter wheat by isolation strips of tall-growing crops? Can we decrease FHB infection and DON contamination of winter wheat by reducing the pre-crop residues of maize or spring wheat on the soil surface due to silage use or by ploughing under the residues of combined crops? Can nitrolime fertilization help to reduce FHB incidence and DON contamination? How do the residue management and the resulting FHB infestation affect grain yield and grain quality of subsequent winter wheat?

#### 3. Material and methods

On the experimental station Ihinger Hof of Hohenheim University two series of field experiments with artificial inoculation were conducted during 1997-99 with wheat. An isolation-strip experiment included strips of tall growing winter rape crops, separating non-inoculated test plots of wheat from inoculated wheat plots. For the main field experiment, maize or spring wheat were planted as pre-crops in rotations with winter wheat and different crop residue treatments were applied: Pre-crops were either harvested for silage or for grain. Subsequently, the residues were either left on the soil surface or ploughed under before sowing the winter wheat. Nitrogen fertilizer was applied to winter wheat either with calcium ammonium nitrate (CAN) or with nitrolime. From randomly sampled ears, the number of infected ears and the number of infected spikelets were counted. The wheat test crops were combined and deoxynivalenol (DON) concentrations of winter wheat

grain were analysed. Furthermore, several technological grain quality traits of winter wheat were determined. Additional residue management greenhouse tests included residues of maize and spring wheat from the field experiment which were incubated in fiberglass-mesh bags at different soil depths and treated equivalent to the main field experiment. The investigations were focused on the *Fusarium* population densities.

#### 4. Results

Results of 2-year isolation-strips field experiments with wheat showed that isolation-strips of winter rape are suitable to substantially reduce the spread of *F. graminearum* spores from artificially inoculated plots. Isolation strips of 2 m width reduced disease incidence on neighbour plots by more than 50% in most cases. A further increase in isolation strip width (4 or 8 m) did improve the isolation effect, but the differences between isolation strip widths were comparatively small. The infection in test plots was not completely eliminated even with 8 m wide strips. Three greenhouse tests of residue management showed that deeper residue incorporation slightly impaired the decomposition process but effectively reduced the *F. graminearum* populations on residues. The application of nitrolime did not affect the decomposition process but reduced the population level of *F. graminearum*. On the contrary, fertilization with calcium ammonium nitrate (CAN) sometimes even reduced residue decomposition but promoted *F. graminearum* populations. Soaking the residues in a fungicide preparation impaired the decomposition process substantially and eliminated *F. graminearum* on the residues completely.

During 2-year residue management field experiments with artificial inoculation of pre-crops, the pre-crop maize did not show any infection symptoms of *Fusarium* foot rot at the stem base, but the ears of spring wheat were clearly infected with *F. graminearum* (on average 11-53%). In general, *F. graminearum* infection of the subsequent test crops of winter wheat was only on a moderate level (on average 6-25%). But there were no significant differences in infection level after either maize or wheat, and the infection level of winter wheat was especially high after maize for silage use in one year. The reductions of FHB incidence due to ploughing or nitrolime application were 27-32% or 31-59% compared with residues remaining on the surface or CAN fertilization, respectively.

Finally, direct effects of crop rotation in conjunction with the management of infested crop residues on grain yield and nutritional or technological grain quality of wheat were an important aspect of the present study. The field experiments showed that the grain contamination with DON was affected by residue management – ploughing the crop residues under obviously reduced DON concentration in comparison with residues left on the soil surface, either after silage or after grain harvest. The silage use of pre-crops improved winter wheat yield moderately

compared with combining. Pre-crops of maize increased 1000-grain weight of subsequent wheat by 4-8%, compared with preceding spring wheat. Independent of residue management, the sedimentation values were within a range from 30-45 ml indicating a good baking quality. Slight differences in protein content and falling number dependent on pre-crops and the management of their residues were not important under applied aspects.

## 5. Conclusion

Isolation-strips of tall-growing winter rape can significantly suspend the dispersal of *F. graminearum* between experimental plots, but infection is not completely eliminated even with 8 m wide strips. The residue management of maize or spring wheat pre-crops offers opportunities to reduce the *F. graminearum* populations on crop residues. This decreases the FHB disease level and the grain contamination with DON of subsequent winter wheat to some degree, even at a moderate FHB infection level. But at that moderate FHB infection level, the residue management hardly influenced wheat grain yield and technological grain quality.

## **Zusammenfassung**

### **1. Einleitung**

Durch *Fusarium graminearum* verursachte partielle Taubährigkeit hat sich weltweit zu einer problematischen Getreidekrankheit entwickelt. Neben Ertragsverlusten führt der Befall zu einer Kontamination des Korngutes mit toxischen Stoffwechselprodukten, insbesondere Deoxynivalenol (DON). Zudem wurde in stark befallenen Partien eine Verminderung der technologischen Korngutqualität beobachtet. *Fusarium*-Epidemien lassen sich auf eine Übertragung durch befallene Vorfruchtrückstände, vornehmlich von Mais- und Weizenbeständen, zurückführen. Als wichtigstes Inokulum gelten Ascosporen, die auf Ernterückständen an der Bodenoberfläche produziert werden. Ein Ansatzpunkt zu einer Verringerung des Infektionsrisikos und damit einer Sicherung von Kornertrag und Kornqualität ist möglicherweise im Rückstandsmanagement der mit *Fusarium* infizierten Ernterückstände von Mais oder Sommerweizen zu suchen.

### **2. Problemstellung**

Die vorliegende Arbeit basiert auf Feldversuchen und Gewächshaus-Brutexperimenten zur Beantwortung folgender Fragen: Kann die Ausbreitung von *F. graminearum* zwischen Winterweizen-Parzellen mittels Isolationsstreifen hochwachsender Pflanzenbestände vermindert werden? Lässt sich eine *Fusarium*-Infektion und die daraus resultierende DON-Kontamination von Winterweizen vermindern, indem Vorfruchtrückstände von Mais oder Sommerweizen an der Bodenoberfläche entweder durch eine Silagenutzung oder durch Unterpflügen reduziert werden? Können Taubährigkeit und DON-Belastung durch eine Kalkstickstoffdüngung reduziert werden? Wie beeinflusst das Rückstandsmanagement der Vorfruchtrückstände und der daraus resultierende *Fusarium*-Befall den Kornertrag und die Kornqualität des nachfolgenden Winterweizens?

### **3. Material und Methoden**

Auf der Versuchsstation Ihinger Hof der Universität Hohenheim wurden zwei je zweijährige Feldversuchsserien mit gezielt inokuliertem Weizen im Zeitraum von 1997-99 durchgeführt. Zur Simulation des natürlichen Befallsverlaufs wurden befallene Haferkörner ausgebracht. Das Isolationsstreifen-Experiment beinhaltete Streifen mit hochwachsendem Winterraps, die nichtinokulierte Weizen-Testparzellen von inokulierten Weizenparzellen trennten. Für das Hauptexperiment wurden Mais oder Sommerweizen als Vorfrüchte vor darauffolgendem Winterweizen angebaut. Eine unterschiedliche Behandlung der Vorfrüchte bzw. ihrer Rückstände erfolgte über die Nutzung entweder als Ganzpflanzensilage oder die Ernte der Körner. Die Ernterückstände wurden vor der Aussaat des Winterweizens entweder an der Bodenoberfläche belassen oder untergepflügt. Eine

Stickstoffdüngung zum Winterweizen erfolgte als Kalkammonsalpeter oder als Kalkstickstoff. An zufällig entnommenen Ähren-Stichproben wurde die Anzahl der infizierten Ähren und Ährchen ausgezählt. Das Korngut der Weizen-Testparzellen wurde bei Reife gedroschen und dessen DON-Konzentration analysiert. Darüber hinaus wurden verschiedene technologische Kornguteigenschaften untersucht. In ergänzenden Gewächshausexperimenten wurde das Management von Mais- und Sommerweizenrückständen modellhaft untersucht. Die Rückstände wurden in Gazebeutel in unterschiedlichen Bodentiefen inkubiert und entsprechend den Feldexperimenten unterschiedlich behandelt. Die Untersuchungen konzentrierten sich hierbei auf die *Fusarium*-Populationsdichten.

#### 4. Ergebnisse

Ergebnisse der zweijährigen Feldversuche mit Isolationsstreifen zeigten, dass Isolationsstreifen mit Winterraps geeignet sind, die Verbreitung von *F. graminearum* ausgehend von gezielt infizierten Parzellen deutlich zu reduzieren. Der Krankheitsbefall in Nachbarparzellen konnte durch 2 m breite Isolationsstreifen in den meisten Fällen um mehr als 50% reduziert werden. Breitere Isolationsstreifen (4 oder 8 m) verbesserten diesen Isolationseffekt, allerdings waren die Unterschiede zwischen unterschiedlichen Streifenbreiten relativ gering. Selbst mit 8 m breiten Streifen konnte eine Infektion der Testparzellen nicht gänzlich verhindert werden.

Drei Gewächshaus-Experimente zu Rückstandsbehandlungen zeigten, dass ein tieferes Eingraben der Rückstände zwar die Abbauprozesse leicht behinderte, aber die *F. graminearum*-Populationen auf diesen Rückständen deutlich reduzierte. Die Anwendung von Kalkstickstoff beeinträchtigte die Abbauprozesse nicht, reduzierte aber die Populationen von *F. graminearum*. Demgegenüber beeinträchtigte eine Düngung mit Kalkammonsalpeter in einigen Fällen den Abbau und förderte zudem die *Fusarium* Pilzpopulationen. Durch Eintauchen der Rückstände in eine Fungizidbrühe wurden die Abbauprozesse drastisch vermindert sowie *F. graminearum* vollständig beseitigt.

Während der zweijährigen Feldexperimente zum Rückstandsmanagement (Hauptversuch) mit gezielter Inokulation der Vorfrüchte, zeigte die Vorfrucht Mais keinerlei *Fusarium*-Symptome an der Stengelbasis während die Ähren des Sommerweizens deutlich mit *F. graminearum* (durchschnittlich 11-53%) infiziert waren. Allgemein war die nachfolgende Testfrucht Winterweizen nur mäßig mit *F. graminearum* infiziert (durchschnittlich 6-25%). Nach Mais oder Sommerweizen konnten keine Niveauunterschiede nachgewiesen werden. Durch Pflügen oder Kalkstickstoffanwendung konnte eine Reduktion des Befalls um 27-32% bzw. 31-59% gegenüber gemulchten Rückständen bzw. einer Düngung mit Kalkammonsalpeter erreicht werden.

Ein wichtiger Aspekt der vorliegenden Studie war die Untersuchung der Vorfruchteffekte in Verbindung mit der Behandlung infizierter Vorfrucht-

Rückstände auf den Kornertrag sowie die ernährungsphysiologische und die technologische Korngutqualität. Die Ergebnisse der Feldversuche zeigten, dass die Kontamination des Korngutes mit DON durch das Management der Rückstände beeinflusst werden kann - ein Unterpflügen der Rückstände reduzierte die DON Konzentration gegenüber gemulchten Rückständen deutlich, sowohl nach einer Silage- als auch nach Kornnutzung. Eine Silagenutzung der Vorfrüchte führte zu einer leichten Ertragssteigerung beim folgenden Winterweizen. Durch die Vorfrucht Mais konnte gegenüber der Vorfrucht Sommerweizen die Tausendkornmasse des nachfolgenden Weizens um 4-8% angehoben werden. Unabhängig von der Rückstandsbehandlung zeigten die Sedimentationswerte innerhalb eines Bereichs von 30-45 ml gute Backeigenschaften an. Anwendungsbezogen waren geringe Unterschiede im Proteingehalt und in der Fallzahl in Abhängigkeit von Vorfrüchten und Rückstandsmanagement von untergeordneter Bedeutung.

## **5. Schlussfolgerung**

Isolationsstreifen mit hoch wachsenden Winterrapsbeständen reduzieren die Verbreitung von *F. graminearum* zwischen Versuchspartzen deutlich, jedoch kann eine Infektion selbst mit 8 m breiten Streifen nicht gänzlich verhindert werden. Über eine gezielte Behandlung ist eine Reduktion von *F. graminearum*-Populationen auf Ernterückständen von Mais und Sommerweizen möglich. Dies vermindert auch bei niedrigem Krankheitsdruck in begrenztem Umfang die Infektion durch *Fusarium* und die DON-Kontamination des Kornguts bei nachfolgendem Winterweizen. Bei solch geringem Krankheitsdruck durch *Fusarium* beeinflusst das Management der Vorfruchtrückstände den nachfolgenden Weizenantrag und die technologische Korngutqualität allerdings kaum.

## H. References

- ANDERSON, A. L.: The development of *Gibberella zeae* head blight of wheat. - *Phytopathology* **38**, 595-611, 1948.
- ANDRÉN, O.: Decomposition of shoot and root litter of barley, lucerne and meadow fescue under field conditions. - *Swed. J. agric. Res.* **17**, 113-122, 1987.
- AUFHAMMER, W., E. Kübler, H.-P. Kaul, W. Hermann, D. Höhn, C. Yi: Infection with head blight (*F. graminearum*, *F. culmorum*) and Deoxynivalenol concentration in winter wheat as influenced by N fertilization. - *Pflanzenbauwiss.* **4** (2), 72-78, 2000.
- AOSA (Association of Official Seed Analysts): Rules for Testing Seeds. - *Proceedings of the Association of Official Seed Analysts* **60** (2), 39, 1978.
- BAIER, W. H., G. BEESE, E. ROICK, E. ALBERT, C. GEBHART, H. ZIMMERMANN: Brauweizenbericht Sachsen - Sorteneignung, Anbauggebiete, Anbaubedingungen. - *Schriftenreihe Sächsische Landesanstalt für Landwirtschaft* **3** (4), 65, 1998.
- BECHTEL, D. B., L. A. KALEIKAU, L. A. GAINES, L. M. SEITZ: The effects of *Fusarium graminearum* infection on wheat kernels. - *Cereal Chemistry* **62** (3), 191-197, 1985.
- BECK, R., A. SÜSS, J. LEPSCHY: *Fusarien* verantwortlich für Bierfehler? - *Pflanzenschutz-Praxis* 2/93, 26-29, 1993.
- BECK, R., J. LEPSCHY, A. OBST: Gefahr aus der Maisstoppel. - *DLG-Mitteilungen* 5/97, 34-38, 1997.
- BECK, R., J. LEPSCHY: Ergebnisse aus dem Fusarium-Monitoring 1989-1999 – Einfluss der produktionstechnischen Faktoren Fruchtfolge und Bodenbearbeitung. - *LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau)* **3**, 39-47, 2000.
- Brown, P. L., D. T. DICKEY: Losses of wheat straw residue under simulated conditions. - *Soil Sci. Soc. Am. Proc.* **34**: 118-121, 1970.
- BSA (Bundessortenamt): Beschreibende Sortenliste - Getreide, Mais, Ölfrüchte, Leguminosen (groszkörnig) und Hackfrüchte (ausser Kartoffeln). - Verlag Landbuch, Hannover, 1997.
- COOK, R. J.: *Fusarium* diseases of wheat and other small grains in North America. In: P. E. Nelson, T. A. Toussoun, R. J. Cook (eds), *Fusarium-Diseases, Biology, and Taxonomy*. - Pennsylvania State University Press, University Park, PA, 39-52.1981.
- DIEHL, T.: Weizenfusariosen - Zur Symptomentwicklung und Schadensanalyse bei Blatt- und Ährenbefall. - PhD Thesis, Univ. Göttingen, Germany, 1984.
- DILL-MACKY, R.: *Fusarium* head blight: recent epidemics and research efforts in the upper midwest of the United States. - In: DUBIN, H. J., L. GILCHRIST, J. REEVES, A. MCNAB (eds.), *Fusarium Head Scab: Global status and future prospects*, CIMMYT, Mexico, 1-6, 1996.



- DILL-MACKY, R., R. K. JONES: The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. - Plant Disease **84**, 71-76, 2000.
- DORAN, J. W.: Tillage effects on microbiological release of soil organic nitrogen. Texas Agric. Exp. Stn. Misc. Publ. College Station, Tx., 1987.
- DOUGLAS, C. L., R. R. ALLMARAS, P. E. RASMUSSEN, R. E. RAMIG, N. C. ROAGER: Wheat straw composition and placement effects on decomposition in dryland agriculture of the Pacific Northwest. - Soil Sci. Soc. Am. J. **44**, 833-837, 1980.
- FEHRMANN, H., T. DIEHL: Partielle Taubährigkeit chemisch behandeln. - Pflanzenschutz-Praxis **2**, 29-31, 1989.
- FERNANDEZ, M. R., J. M. C. FERNANDES: Survival of wheat pathogens in wheat and soybean residues under conservation tillage systems in southern and central Brazil. - Can. J. Plant Pathol. **12**, 289-294, 1990.
- FERNANDO, W. G. D., T. C. PAULITZ, W. L. SEAMAN, P. DUTILLEUL, J. D. MILLER: Head Blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. - Phytopathology **87**, 414-421, 1997.
- GANG, G.: Genetische, umweltbedingte und physiologische Einflüsse auf die Resistenz bzw. Aggressivität im Pathosystem Roggen *Fusarium culmorum* (W. G. Sm) Sacc. - PhD Thesis, Univ. Hohenheim, Stuttgart, Germany, 1997.
- GILGENBERG-HARTUNG, A: Risiken durch Ährenfusariosen. Cyauamid Agrar, 1999.
- GRACIA-GARZA, J. A., D. R. FRAVEL: Effect of relative humidity on sporulation of *Fusarium Oxysporum* in various formulations and effect of water on spore movement through soil. - Phytopathology, **88**, 544-549, 1998.
- HART, P., R. WARD: Efficacy of fungicides on *Fusarium* head blight severity and levels of vomitoxin. - In: Proceedings of National *Fusarium* Head Blight Forum, St. Paul, MN Eagan press, 40-41, 1997.
- HAWARE, M.P., M. P. NENE, M. NATARAJAN: The survival of *Fusarium oxysporum* f. sp. Ciceri in the soil in the absence of chickpea. - Phytopath. Medit. **35**, 9-12, 1996.
- HEISLER, V. C. Significance of microflora, mesofauna and macrofauna for the decomposition of straw in differently compacted arable soils. - Zool. Anz. **233**, 153-172, 1994.
- HERMANN, W., E. KÜBLER, W. AUFHAMMER: Ährenbefall mit *Fusarium* und Toxingehalt im Korngut bei verschiedenen Wintergetreidearten. - Pflanzenbauwissenschaften **2** (3), 97-107, 1998.
- HERMANN, W., W. AUFHAMMER, E. KÜBLER, H.-P. KAUL: Auswirkungen von Ährenbefall mit *Fusarium graminearum* auf die Kornqualität von Winterweizen, Wintertriticale und Winterroggen. - Pflanzenbauwiss. **3**, 82-87, 1999.
- HOCKING, A. D., J. I. PITT: Dicloran-glycerol medium for enumeration of xerophilic fungi from low- moisture foods. Plenum Press, New York and London, S. 315, 1980.

- HOFFER, J. N., A. G. JOHNSON, D. ATANASOFF: Corn-root rot and wheat scab. - J. Agric. Res. **13**, 611-612, 1918.
- HOLBERT, J. R., J. F. TROST, J. N. HOFFER: Wheat scabs as affected by systems of rotation. - Phytopathology **9**, 45-47, 1919.
- HOLLAND, E. A., D. C. COLEMAN: Litter placement effects on microbial and organic matter dynamics in an agroecosystem. - Ecology **68**, 425-433, 1987.
- HUBBARD, V. C., D. JORDAN: Nitrogen recovery by corn from Nitrogen-15 labeled wheat residues and intact roots and soil. - Soil Sci. Soc. Am. J. **60**, 1405-1410, 1996.
- HUBER, D. M., R. D. WATSON: Crop residues, nitrogen, and plant disease. - Soil Sci. **100**, 302-308, 1965.
- HUI, Y., C. K. EVANS, E. K. KOLACZKOWSKI, M. R. DILL, C. J. MIROCHA, Y. HUI: Chemistry, physiology and role of deoxynivalenol in pathogenicity. - Bulletin of the Institute for Comprehensive Agricultural Sciences, Kinki University. **5**, 1-11, 1997.
- JAMES, W. C., C. S. SHIH: Relationship between incidence and severity of Powdery Mildew and Leaf Rust on winter wheat. - Phytopathology **63**, 183-187, 1973.
- JENKINSON, P., D. W. PARRY: Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. - Mycol. Res. **98**, 506-510, 1994a.
- JENKINSON, P., D. W. PARRY: Isolation of *Fusarium* species from common broad leaved weeds and their pathogenicity to winter wheat. - Mycol. Res. **98**, 776-780, 1994b.
- JONES, R. K., C. J. MIROCHA: Quality parameters in small grains from Minnesota affected by *Fusarium* head blight. - Plant Disease **83**, 506-511, 1999.
- JORDAN, V. W. L., J. A. HUTCHEON: Evaluation of an experimental fungicide against *Fusarium* disease of winter wheat in glasshouse and large pot experiments outside. - Report Long Ashton Research Station, 1989.
- KOEHLER, B., J. G. DICKSON, J. R. HOLBERT: Wheat scab and corn root rot caused by *Gibberella saubinetii* in relation to crop successions. - J. Agric. Res. **27**, 861-878, 1924.
- KRÜGER, W.: Mais Krankheiten + Schädlinge und deren Bekämpfung. - Saaten Union GmbH, Hannover, 1985.
- LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau): Ährenfusariosen des Getreides. 2. Auflage. - Freising, Sondernummer **1**, 1997.
- LEPSCHY, J., R. BECK: Vorkommen und Bedeutung von Fusarien und ihren Toxinen im Getreide. - Bodenkultur und Pflanzenbau, Sondernummer **1**, 1997.
- MARTIN, R. A., H. W. JOHNSTON: Effects and control of *Fusarium* diseases of cereal grains in the Atlantic provinces. - Can. J. Plant Pathol. **4**, 210-216, 1982.
- MARTIN, R. A., J. A. MACLEOD, C. CALDWELL: Influences of production inputs on incidence of infection by *Fusarium* species on cereal seed. - Plant Disease **75**, 784-788, 1991.

- MALKOMES, H. -P.: Strohrotteversuche zur Erfassung von Herbizid-Nebenwirkungen auf den Strohumsatz im Boden. - *Pedobiologia*, **20**: 417-427, 1980.
- MCMULLEN, M. P., R. W. Stack: Effects of isolation techniques and media on the differential isolation of *Fusarium* species. - *Phytopathology*, **73**, 458-462, 1983.
- MESTERHAZY, A.: Breeding for resistance to fusarium head blight of wheat. - In: H. J. DUBIN, L. GILCHRIST, J. REEVES, A. MCNAB (eds), *Fusarium Head Scab: Global status and future prospects*. - International Maize and Wheat Improvement Center, 79-85, 1996a.
- MESTERHAZY, A.: Fungicide control of fusarium scab and impact on toxin contamination. - In: H. J. DUBIN, L. GILCHRIST, J. REEVES, A. MCNAB (eds), *Fusarium Head Scab: Global status and future prospects*. - International Maize and Wheat Improvement Center, 120-124, 1996b.
- MIEDANER, T.: Entwicklung von Methoden zur Bestimmung der Fusarienresistenz in frühen Wachstumsstadien des Weizens. - PhD Thesis, Univ. Hohenheim, Stuttgart, Germany, 1986.
- MIEDANER, T.: Review: Breeding wheat and rye for resistance to *Fusarium* diseases. - *Plant Breeding* **116**, 201-220, 1997.
- MIELKE, H.: Untersuchungen über *Fusarium culmorum* (W. G. Sm) Sacc. als Fuss- und Blattkrankheitserreger beim Weizen. - *Mitt. Biol. BundAnst. Ld. - u. Forstw.* **238**, 1-101, 1988.
- MILLER, J. D.: Epidemiology of *Fusarium* ear diseases of cereals. In: MILLER JD and TRENHOLM HL (eds), *Mycotoxins in grain*, Eagan Press, St. Paul, MN. 19-36, 1994.
- MILLER, J. D., J. CULLEY, K. FRASER, S. HUBBARD, F. MELOCHE: Effect of tillage practice on *Fusarium* head blight of wheat. - *Can. J. Plant Pathol.* **20**, 95-103, 1998.
- MILUS, E. A., C. E. PARSONS: Evaluation of folia fungicides for controlling *Fusarium* head blight of wheat. - *Plant Disease* **78**, 697-699, 1994.
- MIROCHA, C. J., H. K. ABBAS, C. E. WINDELS, W. XIE: Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. - *Applied and Environmental Microbiology* **55**, 1315-1316, 1989.
- MOLLENHAUER, S., H. PESCHKE, A. FLAATTER: Abbauverhalten von fungizidbehandeltem Weizenstoh. - *Agribiol. Res.* **52**, 3-4, 1999.
- MÜLLER, H.-M., K. SCHWADORF: A survey of the natural occurrence of *Fusarium* toxins in wheat grown in a southwestern area of Germany. - *Mycopathologia* **121**, 115-121, 1993.
- NYVALL, R. F.: Chlamydospores of *Fusarium roseum* 'Graminearum' as survival structures. - *Phytopathology*, **60**, 1175-1177, 1970.

- OBST, A.: Wie man Ährenfusariosen vermeidet. - DLG-Mitteilungen **50**, 470-471, 1988
- OBST, A., J. LEPSCHY-V. GLEISSENTHAL, G. HUBER: Zur gezielten Bekämpfung der Ährenfusarien bei Weizen - Beobachtungen und Versuchsergebnisse aus Bayern. - Gesunde Pflanzen **44**, 40-47, 1992.
- Obst, A.: Untersuchungen zur Epidemiologie und Bekämpfung des Ährenparasiten *Fusarium graminearum* an Weizen. - Mitt. Biol. Bundesanst. Land- und Forstwirtsch. **301**, 73, 1994.
- Obst, A.: Zu Epidemiologie und Bekämpfung von *Fusarium graminearum* und *F. culmorum*. - LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau) **1**, 2-28, 1997.
- OBST, A., P. GAMMEL: Fungizide gegen den Ährenparasiten *Fusarium graminearum*. - LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau) **3**, 89-98, 2000.
- PARRY, D. W., P. JENKINSON, L. MCLEOD: *Fusarium* ear blight (scab) in small grain cereals - a review. - Plant Pathol. **44**, 207-238, 1995.
- REIS, E. M. Integrated disease management - The changing concepts of controlling head blight and spot blotch. In: Saunders DA (ed) Wheat for the nontraditional warm areas. Proc. Int. Conf. CIMMYT, Mexico, D. F. 165-177, 1990.
- RINTELEN, J.: Die Fusarienflora in Maisstopplern. - LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau) **1**, 48-49, 1997.
- RINTELEN, J.: Zeitpunkt der Fusarieninfektion der Ähre. - LBP (Bayerische Landesanstalt für Bodenkultur und Pflanzenbau) **3**, 89-98, 2000.
- SAS Institute Inc.: SAS/STAT user's guide, version 6, fourth edition. - Cary, NC, USA, 1989.
- SCHWADORF, K., H.-M. MÜLLER: Determination of trichothecenes in cereals by gas chromatography with ion trap detection. - Chromatographia **32**, 137-142, 1991.
- SCHWEYDA, H. J.: Pathogene und fakultativ pathogene samenbürtige Pilze auf Saatgetreide aus Baden-Württemberg: Biologie - Befallssituation - Konsequenzen. - PhD Thesis, Univ. Karlsruhe, Germany, 1996.
- SCOTT, N. A., C. V. COLE, E. T. ELLIOTT, S. A. HUFFMAN: Soil textural control on decomposition and soil organic matter dynamics. - Soil Sci. Soc. Am. J. **60**, 1102-1109, 1996.
- SEAMAN, W. L.: Epidemiology and control of mycotoxigenic fusaria on cereal grains. - Can. J. Plant Path. **4**, 187-190, 1982.
- SMILEY R. W., R. J. COOK, R. I. PAPPENDICK: *Fusarium* foot rot of wheat and peas as influenced by soil applications of anhydrous ammonia and ammonia potassium azide solutions. - Phytopathology **62**, 86-91, 1972.
- SNIJDERS, C. H. A., J. PERKOWSKI: Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. - Phytopathology **80**, 566-570, 1990.

- SNIJERS, C. H. A.: Breeding for resistance to *Fusarium* in wheat and maize. - In: MILLER, J. D., H. L. THRENHOLM (eds.), *Mycotoxins in grain-compounds other than aflatoxin*, St Paul, MN Eagan press, 37-58, 1994.
- SNYDER, W. C., S. M. NASH: Relative incidence of *Fusarium* pathogens of cereals in rotation plots at Rothamsted. - *Trans. Br. Mycol. Soc.* **51**, 417-425, 1968.
- STRAUSBAUGH, C. A., O. M. MALOY: *Fusarium* scab of irrigated wheat in Central Washington. - *Plant Disease* **70**, 1104-1106, 1986.
- STURZ, A.V., C. C. BERNIER: Survival of cereal root pathogens in the stubble and soil of cereal versus noncereal crops. - *Can. J. Plant Pathol.* **9**, 205-213, 1987.
- STURZ, A. V., H. W. JOHNSTON: Characterization of *Fusarium* colonization of spring barley and wheat produced on stubble or fallow soil. - *Can. J. Plant Path.* **7**, 270-276, 1985.
- Su, H., A. H. C. VAN BRUGGEN, , K. V. SUBBARAO: Spore release of *Bremia lactucae* on lettuce is affected by timing of light initiation and decrease in relative humidity. - *Phytopathology*, **90** (1): 67-71, 2000.
- SUMMERELL, B. A., L. W. BURGESS, T. A. KLEIN, A. B. PATTISON: Stubble management and the site of penetration of wheat by *Fusarium graminearum* group 1. - *Phytopathology* **80**, 877-879, 1990.
- SUTTON, J. C.: Epidemiology of wheat head blight and maize ear rot caused by *F. graminearum*. - *Can. J. of Plant Pathol.* **4**, 195-209, 1982.
- SUTTON, J. C., T. J. VYN: Crop sequences and tillage practices in relation to diseases of winter wheat in Ontario. - *Can. J. of Plant Pathol.* **12**, 358-368, 1990.
- TANAKA, T., S. YAMAMOTO, A. HASEGAWA, N. AOKI, J. R. BESLING, Y. SUGIURA: A survey of the natural occurrence of *Fusarium* mycotoxins, deoxynivalenol, nivalenol and zearalenone, in cereals harvested in the Netherlands. - *Mycopathologia* **110**, 19-22, 1990.
- TEICH, A. H., K. NELSON: Survey of *Fusarium* head blight and possible effects of cultural practices in wheat fields in Lambton County in 1983. - *Can. Plant Dis. Surv.* **64**, 11-13, 1984.
- TEICH, A. H.: Less wheat scab with urea than with ammonium nitrate fertilizers. - *Cereal Res. Commun.* **15**, 35-38, 1987.
- TEICH, A. H.: Epidemiology of wheat (*Triticum aestivum* L.) scab caused by *Fusarium* spp. - In: CHELKOWSKI, J. (ed.), *Fusarium Mycotoxins, Taxonomy and Pathogenicity*, Elsevier, Amsterdam, 269-282, 1989.
- WEINERT, J., G. A. WOLF: Gegen Ährenfusarien helfen nur resistente Sorten. - *Pflanzenschutz-Praxis* **2**, 30-32, 1995.
- WILCOXSON, R. D., R. H. BUSCH, E. A. OZMON: *Fusarium* head blight resistance in spring wheat cultivars. - *Plant Disease* **76**, 658-661, 1992.
- WANG, Y. Z.: Epidemiology and management of wheat scab in china. In: H.J. DUBIN, L. GILCHRIST, J. REEVES, A. MCNAB (eds), *Fusarium Head Scab: Global*

- status and future prospects. - International Maize and Wheat Improvement Center, 97-105, 1996.
- WEINERT, J, G. A. WOLF: Erfahrungen zum Auftreten von Ährenfusariosen und Bekämpfungsstrategie im Weizen. - *Fusarium*-Symposium an der Universität Hohenheim. 18-19, 1999.
- WHITMORE, A. P., E. HANDAYANTO: Simulating the mineralization of N from crop residues in relation to residue quality. In: CADISCH G. and GILLER K.E. (eds), Driven by nature. Plant litter quality and decomposition. CAB International, Wallingford (UK), 337-348, 1997.
- WIESE, M. V.: Scab (head blight). In: Compendium of Wheat Diseases. 2<sup>nd</sup> ed. American Phytopathological Society, St. Paul, MN. 16-18, 1987.
- WOLTZ, S. S., A. W. ENGELHARD: *Fusarium* wilt of chrysanthemum: Effect of nitrogen source and lime on disease development. - *Phytopathology* **63**, 155-157, 1972.
- YU, H., C. K. EVANS, E. K. KOLACZKOWSKI, M. R. DILL, C. J. MIROCHA, Y. HUI: Chemistry, physiology and role of deoxynivalenol in pathogenicity. - Bulletin Institute for Comprehensive Agricultural Sciences, Kinki University, **5**, 1-11, 1997.

## I. Acknowledgments

Foremost I wish to thank Prof. Dr. W. Aufhammer for offering me the opportunity of this study. Thanks for his scientific advice, valuable discussions, and creative suggestions during every stage of the work.

I am most grateful to Dr. H.-P. Kaul and Dr. E. Kübler for their scientific support and guidance in research and in preparing this thesis.

Thanks to the colleagues in our research group: Dr. A. Rosenberger, Dipl.-Ing.sc.agr. L. Weik, Dipl.-Biol. D. Höhn, Mrs. A. Kämpf, Mrs. B. Beierl and Mrs. A. Richter for creating a pleasant working environment, and their friendship and technical assistance.

The technical support of the DON analyses by the State Institute for Agricultural Chemistry at the University of Hohenheim, namely by Prof. Dr. H. Schenkel, Dr. B. Eckstein, Dr. K. Schwadorf and Mrs. A. Eschle., as well as by the Institute for animal nutrition at the University of Hohenheim, namely by Dr. M. Schollenberger, is gratefully acknowledged.

Special thanks to Dr. G. Renz, Mr. H. Kärcher, Mr. M. Zahner and Mr. H. Grözingen and other people at the experimental station Ihinger Hof of Hohenheim University for their excellent and efficient assistance in completing my field and greenhouse experiments as well as the analyses of grain quality.

I also like to thank all other colleagues at the Institute.

Finally I will give my thanks to Prof. Dr. V. Römheld and Prof. Dr. F.S. Zhang for offering me the opportunity to come to Germany.

Cuilin Yi  
Universität Hohenheim  
Stuttgart, Germany  
2001